RELATIONSHIPS OF SOILBORNE MICROBIAL COMMUNITIES TO INFECTION OF ROOT SYSTEMS OF TOBACCO BY *PHYTOPHTHORA PARASITICA* VAR *NICOTIANAE*

By

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By

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Major Department: Plent Pethology

Infectione of tobecco roote by Phytophthore peresitice ver. nicotiene and development of bleck shank were evelueted in reletion to behevior of eeverel pathoeyetem componeets. Develepment of root systeme wee characterized under contrelled environmentel conditioee in e plent growth roem by use of morphometric root enelyeis. Patterns of development were eimiler for root systems of a susceptible end resietent cultiver in steble end diarupted soil ecesystems represented by rew end autocleved soils, reepectively.

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[illegible]e pathogen were observed on root systems of both cultivars exposed to 50 chlamydospores per gram of soil for 2 weeks in both ecosystems. Average numbers of infected roots per infected seedling varied between 5.4 and 16.1, more than 80% of infections occurred on first-order roots, which were defined as those terminating in apical meristems. Development of root systems was not altered significantly by infection during the 2 weeks of growth in infested soil.

Communities of microorganisms developed more rapidly and with greater diversity in association with tobacco root systems grown in raw soil as compared to those grown in autoclaved soil. Fungi colonized surfaces of first-order roots more densely and extensively in raw soil than in autoclaved soil. A composite of organisms which colonized tobacco roots rapidly in raw soil was evaluated for its ability to compete with the pathogen for occupation of sites susceptible to infection within root systems. Amendment of soils with a composite comprised of propagules of Trichoderma harzianum, Aspergillus carbonarius, Aspergillus terreus, Penicillium steckii, and Pseudomonas putida did not reduce significantly the numbers of early root infections. Soil amendment with the composite was associated with increases in densities of fungi and fluorescent Pseudomonas spp. around tobacco roots as determined from plate counts; however, no alterations in degree of root surface coverage by fungi were observed. Survival of the pathogen in

non-rhizosphere soil was not influenced by [illegible] the composite. Amendment of infested soils with the composite was associated with decreased mortalities of tobacco after 30 days of plant growth in the glasshouse.

CHAPTER 1
INTRODUCTION

Black shank is a serious disease of tobacco (Nicotiana tabacum L.) incited by the soilborne pathogen, Phytophthora parasitica Dast. var. nicotianae (Breda de Haan) Tucker. This pathogen produces several types of spores, including chlamydospores, sporangiospores, and zoospores. Zoospores are able to germinate and infect tobacco plants either at points within root systems or along the lower stem at or near the soil line (40, 50, 65). The ability of other types of spores to infect various tissues of tobacco plants directly has not been documented completely. However, infections of several plant hosts by species of Phytophthora have been observed in soils infested with chlamydospores of these pathogens (36, 39, 40, 62, 83, 84).

A number of environmental factors have been observed to influence the behavior of Phytophthora spp. Physical factors such as light (9, 15, 32), temperature (9, 19, 36, 79), soil water potential (17, 18, 79), and aeration (63, 64), have been shown to influence the growth and reproduction of these fungi as well as their pathogenic behaviors. Organic substrate composition (16, 61) and activities of surrounding soilborne microorganisms (12, 55, 57, 96) also have been observed to influence pathogen development and behavior.

Residual inoculum of P. parasitica var. nicotianae consists predominantly of chlamydospores, which occur either freely in soil or within infested plant debris (40, 62). Initial populations of the pathogen in soil are extremely low and highly aggregated (20, 39), but they may increase rapidly within the rhizosphere of tobacco plants (22, 33). Secondary inoculum is produced within this region after root infection and may consist of sporangia, zoospores, chlamydospores, or possibly oospores. With the possible exception of oospores, each of these forms of inoculum is capable of either reinfecting the original host plant or infecting nearby, noninfected plants. The potential for rapid increases in populations of P. parasitica var. nicotianae in association with tobacco roots and subsequent inoculum dissemination presents a great obstacle to the control of black shank by manipulations of antagonistic microorganisms.

Incidence of infection of tobacco has been evaluated in relation to initial inoculum densities of some of these types of spores (40, 78); patterns of infections on individual root systems, however, have not been elucidated. In particular, the susceptibilities of various root tissues to infection by this pathogen have not been evaluated under controlled conditions.

A variety of microorganisms has been reported to be antagonistic to species of Phytophthora (51, 67, 94). Many types of bacteria and fungi have been found in association

Like degraded mycelia and other structures of these pathogens in field soils. A number of these organisms, as well as others recovered from non-rhizosphere soil or the rhizospheres of plants, have been shown to produce metabolites which are deleterious to vegetative growth of Phytophthora spp in vitro. Additionally, observations of soils suppressive to Phytophthora spp. have been reported on many occasions (51); however, few identifications of specific organisms or factors responsible for suppression have been made.

Despite these suggestions of availability of antagonists, few attempts have been made to manipulate organisms directly to control diseases caused by Phytophthora spp. Effective control of tobacco black shank by introductions of antagonists is dependent upon an adequate understanding of the biology of P. parasitica var. nicotianae in non-rhizosphere soil and in association with tobacco plants. Since initial inoculum of the pathogen in soil is sparse, it is unlikely that sufficient interactions could be encouraged economically between populations of the pathogen and introduced antagonists to reduce inoculum densities of P. parasitica var. nicotianae to significantly lower levels. Alternatively, important opportunities for interactions between these populations exist within the tobacco rhizosphere, since this is the region in which the pathogen is biologically very active but vulnerable. Emphasis on this region also reduces the volume of soil to be manipulated in control efforts.

Effective manipulation of introduced antago[illegible] the rhizosphere of tobacco also requires an understa[illegible] the behaviors of those antagonists within that region. I[illegible] le especially important in relation to the development of tobacco root systems. The importance of host root growth in relation to the development of epidemics involving soilborne pathogens has been discussed previously (35, 45).

Detailed descriptions of root growth have been provided for very few plant species. Root growth of tobacco, in particular, has been described only in terms of weight increase over time and in the form of schematic diagrams (68). No information is available as regards the dynamics of formation of tobacco root tissues susceptible to infection by P. parasitica var. nicotianae.

These studies were established to quantify the development of root systems of tobacco during early seedling growth under controlled conditions. Colonization of these roots by P. parasitica var. nicotianae and other members of the surrounding soil microbial community also was evaluated. A number of fungal and bacterial isolates were selected on the basis of their abilities to colonize tobacco root systems rapidly and stably over time. These isolates were combined and evaluated for their ability, as a community, to occupy niches of importance to the pathogen and to reduce infections of tobacco roots and subsequent black shank development.

CHAPTER II
RELATIONSHIPS BETWEEN THE DEVELOPMENT OF ROOT SYSTEMS OF TOBACCO AND INFECTION BY PHYTOPHTHORA PARASITICA VAR. NICOTIANAE

Introduction

Incidence of black shank of tobacco (Nicotiana tabacum L.) has been shown to be related to initial density and aggregation of inoculum of the soilborne pathogen, Phytophthora parasitica Dast var. nicotianae (Breda De Haan) Tucker (11, 20, 40). These relationships have been demonstrated in trials conducted in plant growth rooms and in the field. Incidences of infection or disease have been utilized as measures of the outcome of numerous cycles of interactions which occur between populations of tobacco and this pathogen in the course of black shank epidemic development. More detailed evaluations of patterns of initial infections and progressive colonization of individual root systems by the pathogen would provide greater insight into events associated with disease development in populations of plants. This would provide information on which strategies of disease control could be based.

Estimates of levels of infection of individual plant root systems at defined inoculum densities have been provided for pinto bean, infected by Fusarium solani f. sp.

phaseoli, alfalfa, infected by Fusarium spp., Rhizoc[illegible] spp., and Pythium spp., and peanut, infected by Cylindrocladium crotalariae (15, 30, 89). These estimates have been reported in terms of infections per unit length of root. Unfortunately, the types of roots infected have not always been defined clearly and the significance of particular patterns of infection could not be assessed in terms of tissue susceptibility.

Generally root system development has been evaluated at single points in time using a descriptive scheme based on chronological order of appearance of roots (4, 74). Problems in interpretation of infection patterns may be related to such visualizations of root system structure. Interpretation of infection patterns might be enhanced by the utilization of a scheme of root system development relevant to dynamic analyses of interactions with pathogen populations.

Efforts to provide such detailed descriptions for infection of tobacco in field situations have been hampered by the extremely low initial densities of inoculum of P. parasitica var. nicotianae in soil. Development of extensive root systems is likely to occur before susceptible tissues contact propagules of the pathogen, evaluations of patterns of root infection under these conditions would be virtually impossible with present technology.

In this study infections of individual root systems of tobacco were assessed with a defined inoculum density of P. parasitica var. nicotianae in short term trials in a controlled environment within a plant growth room. Patterns of infection were assessed in relation to various root tissues as defined in a quantitative root analysis system.

Materials and Methods

Blichton sand was passed through a 1 mm sieve and used in all trials. Initial trials were established in a plant growth room to assess patterns of development of root systems of tobacco cultivars susceptible and resistant to P. parasitica var. nicotianae. Two-week-old seedlings of the susceptible cultivar, Hicks, and the resistant cultivar, Speight G-28, were transplanted individually into 100-ml, polypropylene beakers containing 80 g of raw or autoclaved field soil layered over approximately 15 g of autoclaved builder's sand. Autoclaved soil was treated for 1 hour on each of two successive days. Three small holes were made in the bottom of each beaker to provide drainage.

Plants were maintained in watering trays and covered with clear plastic to minimize plant desiccation during 15 days of growth at 25±2 C and 16 hours of light (700 μEin/m^2/sec) per day. Seedlings were watered from below

[illegible] alternate days.

Every 3 days, five seedling root [illegible] tobacco cultivar were removed gently from both raw and autoclaved soils. Each root system was spread carefully on acetate film to expose all roots. The numbers and lengths of root elements in defined root classes were recorded using the Micro-comp data acquisition system developed by Southern Micro Instruments, Inc. (Atlanta, GA 30348). Root elements were as defined in the classification scheme established in the morphometric root analysis system described by Fitter (21). Within this scheme root branching is defined from apical meristems inward (Fig. 2-1). Any root which terminates in an apical meristem is defined as a first-order root. Where two first-order roots merge, there begins a second-order root. Where two second-order roots merge, there begins a third-order root and so forth. The union of a particular root element with that of a higher order does not alter the classification of the element of the latter root order. A characteristic of this system is that both the numbers and lengths of elements in each root order change with time as branching along first-order roots proceeds; first-order root tissues become part of second-order and higher-orders as further branching occurs toward the apical meristem. Within this dynamic scheme, root systems are divided into regions of increasing tissue maturity which correspond to increasing

root order. Root growth trials were conducted [illegible] re
averages of seedling measurements from combined trials were
utilized to derive estimates of parameters describing
dynamics of root system development and patterns of
branching.

Patterns of early root infections of susceptible and
resistant tobacco plants by P. parasitica var. nicotianae
were evaluated in short term trials. Isolate P-230 of the
pathogen, received originally from the Department of Plant
Pathology of the University of California at Riverside, was
utilized in all infection trials. Cultures were maintained
on cornmeal and V-8 juice agars and were transferred
monthly. Chlamydospores of this pathogen were produced
axenically in liquid culture by the method of Tsao (90).
Chlamydospore inoculum, free of viable mycelium, was
prepared according to the method of Ramirez and Mitchell
(73). Concentrations of chlamydospores in the resulting
suspensions were determined from counts of propagules in 20
haemocytometer fields.

Suspensions of chlamydospores were added to both raw
and autoclaved field soils to establish inoculum densities
of 50 chlamydospores per gram of soil. It had been
determined previously that more than 90% of tobacco
seedlings became infected after 14 days of growth in soil
infested at this inoculum level. Infested soil was added
to 100-ml, polypropylene beakers according to the infested
soil layer method of Kannwischer and Mitchell (40).

Sixty-five grams of soil infested with the pathogen were layered over approximately 15 g of autoclaved builder's sand. A final layer consisting of 35 g of either raw or autoclaved, noninfested field soil was placed on the infested soil layer. This procedure was used to allow undamaged root growth of tobacco plants from the upper noninfested soil layer into the infested layer of soil below. A two-week-old seedling of Hicks or Speight G-28 tobacco was transplanted into the noninfested soil layer. Fifteen seedlings of each tobacco cultivar were transplanted in this manner into beakers of both raw and autoclaved soils. Control treatments consisted of six, 2-week-old seedlings transplanted singly into polypropylene beakers containing raw or autoclaved soil which had not been infested with the pathogen. Transplanted seedlings were placed in watering trays, covered with clear plastic, and grown in a plant growth room at 25±2 C and under 16 hours of light (700 μEin/m^2/sec) per day. Plants were watered from below on alternate days.

After 2 weeks of growth, 15 asymptomatic seedlings of each tobacco cultivar were removed gently from both raw and autoclaved, infested soils. Tops of seedlings were removed and root systems were surface-disinfested by dipping in 70% ethanol and rinsing in three changes of deionized water. Each root system was dissected completely according to the classification scheme of the morphometric root analysis system (Fig. 2-1). Roots were plated individually onto a

selective medium containing 10 mg pimaricin (Delvocid, 50% a.i., Gist-Brocades, N. V., Delft, Holland), 250 mg ampicillin (sodium salt, 100% a.i., Sigma Chemical Co., St. Louis, MO 63178), 10 mg rifampicin (Rifamycin SV, 100% a.i., Sigma Chemical Co.), 100 mg pentachloronitrobenzene (Terraclor, 75% a.i., Olin Mathiesen Chemical Corp., Little Rock, AR 72203), 50 mg hymexyzol (Hymexazol, 99.4% a.i., Saukyo Co., LTD., Tokyo, Japan), and 17 g Difco cornmeal agar (Difco Laboratories, Detroit, MI 48201) in 1.0 liter of deionized water (38). After incubation for 48 hours in the dark at 25 C, roots were examined for the emergence of colonies of P. parasitica var. nicotianae.

Patterns of root system branching were evaluated for both tobacco cultivars in raw and autocleved soils. The numbers and lengths of elements in each root order were determined for each of six infected seedlings of each tobacco cultivar grown in infested, raw or autoclaved soil. Numbers and lengths of roots were determined as well for each of six healthy seedlings of each tobacco cultivar grown in each noninfested soil. Trials established to evaluate patterns of early root infection were conducted twice.

Influences of treatments on infection and host root system development were evaluated by analysis of variance within each trial. Since contrasts were selected after examining experimental outcomes, appropriate contrasts between treatments within individual trials were made using

Scheffe's intervals (25). Comparisons of corresponding treatment effects between trials were made using Student's two-sample t test (25).

Susceptibilities of various tissues within individual root systems to infection by *P. parasitica* var. *nicotianae* were evaluated in point inoculation trials. Zoospores of the pathogen were produced by the method of Kannwischer and Mitchell (40). Suspensions of zoospores in a solution buffered with 10^{-4}M 2-(N-morpholino)-ethanesulfonic acid at pH 6.2 were diluted to provide an average of 16 zoospores per 20-µl drop.

Two-week-old Hicks tobacco seedlings were placed on microscope slides which had been covered with a layer of twice-autoclaved soil. Seedlings were grown on soil-coated slides in an incubator at 28 C in 16 hours of light (300 µEin/m^2/sec) per day for an additional 2 weeks. At that time zoospores in a single microdrop were applied either just behind the root tip, 2 cm behind the root tip but still on first-order root tissue, or on second-order root tissue of one seedling. A small piece of parafilm had been placed under the root at each point of inoculation to ensure the stability of the droplet. Inoculated seedlings were placed in moist chambers and incubated for 4 hours prior to covering inoculation points with moistened, autoclaved soil. Seedlings then were returned to the incubator and after 48 hours of incubation, inoculated roots were excised from seedlings. Roots were dipped

briefly in 0% ethanol, rinsed three times in deionized water, blotted dry and plated on the selective medium. Plates were incubated for 48 hours in the dark at 25 C and examined for emergence of colonies of P. parasitica var. nicotianae from inoculated root tissues. Trials were conducted three times. Within each trial inoculations at each of the three points on root systems were replicated ten times. Average percentages of infection at each point within three trials were transformed by arcsine square-root and compared by Tukey's multiple comparison procedure for honestly significant differences (25).

Results

During 15 days of growth in a plant growth room, the numbers and total lengths of elements within different root orders of Hicks and Speight G-28 tobacco increased in similar fashions in two trials. To provide estimates of parameters of root growth which might be encountered in a number of experiments, data from both trials were combined prior to analysis. The increases in numbers and total lengths of elements of first-order and second-order roots in raw or autoclaved soil over time were described well by an exponential function. When curves describing these relationships were linearized by use of the natural log transformation, coefficients of determination were always

greater than 0.94. Typical of the patterns of [illegible] observed was that of average numbers of elements of first-order, second-order, and third-order roots of Hicks tobacco during growth in autoclaved soil (Fig. 2-2). Similar average numbers of elements of each root order were observed for each tobacco cultivar in both soil ecosystems at each sampling date. At the end of 15 days of growth, the average numbers of elements of first-order roots for Hicks or Speight G-28 tobacco seedlings varied between 37 and 56 roots per plant (Table 2-1). The average total lengths of first-order roots varied between 92 and 122 cm for either cultivar in either soil ecosystem (Table 2-2). Despite such variability there were no significant differences detected in the average numbers or total lengths of elements of first-order or any other order roots in association with either tobacco cultivar or soil ecosystem. Throughout the growth period the total lengths of elements of first-order roots accounted for a minimum of 85% of the average total seedling root lengths.

By the end of the 15-day growth period, root systems of some tobacco plants had branched sufficiently to form third-order and fourth-order roots (Table 2-1). Root elements within these orders did not form until at least 6 or 9 days after seedling transplant. Generally only one or two third-order roots were observed per Hicks or Speight G-28 tobacco seedling after 15 days of growth. A maximum of one fourth-order root was observed for any seedling by

that trend. In contrast to the exponential increases in total lengths of elements within first-order and second-order root classes, average lengths of roots in each root order increased much more slowly (Table 2-3).

Further evaluations were made of the rates of increases in numbers and total lengths of elements of first-order and second-order roots for tobacco seedlings with time (21). The exponential curves which described increases in numbers and total lengths of root elements over time were linearized by use of the natural log transformation. The slopes of the linearized curves relating numbers of elements in each root order (N) to time (t) were defined as the relative multiplication rates of elements in each root order:

$$1/N(dN/dt) \quad (no./no./day).$$

Similarly the slopes of the linearized curves relating total root lengths in each root order (L) to time were defined as the relative extension rates of elements in each root order:

$$1/L(dL/dt) \quad (cm/cm/day).$$

The relative multiplication rates of elements of first-order and second-order roots of either cultivar were similar during growth in raw and autoclaved soils (Table

Rates of multiplication always were slightly less [illegible] second-order roots than for first-order roots of either tobacco cultivar during growth in either soil ecosystem. The rates of root length extension for elements of first-order and second-order roots were more variable. Relative extension rates of elements of first-order roots were greater than rates of extension of elements of second-order roots of Hicks tobacco during growth in raw or autoclaved soil. The relationship between extension rates of first-order and second-order roots was reversed in the case of Speight G-28 tobacco seedlings grown in either soil ecosystem. The relative rates of total seedling root length extension were very similar to corresponding rates of extension of elements of first-order roots. Rates of root extension also were evaluated in terms of average rates of extension per root element (cm/root/day) rather than in relation to existing root length (cm/cm/day) (Table 2-2). Within the morphometric root analysis system, this was termed the apparent unit extension rate or the rate of extension per element per root order (21). These rates were derived from a combination of parameters of root growth as follows

$$1/N(dL/dt) \quad \text{(cm/root/day)}$$

Apparent unit extension rates of root elements of first-order roots were more variable than were relative extension rates of elements in this order (Table 2-2). Maximum and minimum rates of 0.95 and 0.65 cm/root/day occurred with Hicks tobacco grown in raw soil and Speight G-28 tobacco grown in autoclaved soil, respectively. The different values of the rates were not correlated with significant differences in total lengths of first-order roots of the two tobacco cultivars in either soil ecosystem. Apparent unit extension rates of elements of second-order roots also were variable between cultivars and soil ecosystems. The apparent unit extension rates of second-order roots were less than extension rates of first-order roots for Hicks tobacco in autoclaved and raw soils. Apparent unit extension rates of second-order roots were greater than those of first-order roots of Speight G-28 tobacco in both soils. Rates of root length increase were not derived for third-order or fourth-order roots because elements within these orders appeared too late in the trial period to provide sufficient values for calculations.

After 14 days of growth in raw or autoclaved soil infested with P. parasitica var. nicotianae, a minimum of 67% of tobacco seedlings were infected by the pathogen, in most cases more than 87% of the tobacco population was infected. At the end of the growth period only one or two seedlings in any treatment combination had died from black

shank. All of these seedlings exhibited symptoms typical of black shank, but they were not evaluated further in this study. Previously, however, direct microscopic observation of stained root systems of such seedlings had revealed that first-order roots within the vicinity of the root crown, as well as the lower stem tissues themselves, always had been colonized by the pathogen (English, unpublished).

Within each of two trials, no significant differences were observed in the average numbers of infected roots per infected Hicks or Speight G-28 tobacco seedling in raw or autoclaved soil (Table 2-4). The average numbers of infected roots observed per infected seedling ranged from 10.1 to 16.1 and from 5.4 to 12.3 in trial 1 and trial 2, respectively. Typically between 1 and 31 infected roots were observed on any single infected root system. The numbers of infected roots observed per infected seedling varied between trials only in association with Speight G-28 tobacco grown in autoclaved, infested soil ($p=0.05$). More than 80% of all infections per infected seedling of either tobacco cultivar occurred on first-order roots.

The efficiency of inoculum of a pathogen describes the proportion of propagules that infect roots. Within the present study the number of chlamydospores added to a defined amount of soil was controlled. The efficiency of inoculum for observed infections therefore was defined as the ratio of the total number of infected roots observed per tobacco root system to the total number of

chlamydospores added to the volume of soil in which each plant was grown. The average efficiencies of chlamydospores of this pathogen for observed infections of both tobacco cultivars in raw and autoclaved soil were very low in both trials and varied between 0.002 and 0.005 (Table 2-4). Within each trial average inoculum efficiencies did not vary significantly in association with either cultivar in either soil ecosystem. Efficiency varied significantly between trials only in association with Speight G-28 tobacco plants grown in autoclaved, infested soil ($p=0.05$).

During 14 days of growth in infested soils, the development of root systems of tobacco was not altered significantly by infection with F. _parasitica_ var. _nicotianae_. In particular, within each trial there were no significant differences observed between the numbers (Table 2-5) or total lengths (Table 2-6) of elements of first-order or second-order roots per infected or healthy seedling associated with either cultivar grown in either soil ecosystem. Comparisons of growth of third-order and fourth-order roots were not made as elements of these orders had just begun to appear. Significant differences in numbers or total lengths of elements of first-order and second-order roots were observed sporadically between trials within corresponding treatment combinations ($p=0.05$). The most noticeable differences between trial 1 and trial 2 as regards these root growth characteristics

were observed in associat[illegible] Hicks tobacco seedlings grown in raw or autoclaved soil which had not been infested with chlamydospores of the pathogen. The average lengths of root elements per first-order or second-order root did not vary significantly in association with any treatment combinations within or between trials (Table 2-7). Average lengths of fourth-order roots were equal to zero in some treatments because elements in this order had not yet formed.

To evaluate the contribution of host plant growth to the development of an epidemic, control of that component of a pathosystem must be achieved consistently in repeated trials. The consistency of root system development of tobacco over repeated trials was evaluated by comparing root systems of each cultivar in the infection trials to predicted root system development as estimated from results of the earlier time course trials. Within the earlier trials exponential curves describing the time-related increases in numbers and total lengths of first-order and second-order roots per seedling were transformed using the natural log transformation. First-order linear equations derived by regression analyses described relationships between transformed values and time very well. By interpolation, estimates were made of the expected mean numbers and total lengths of first-order and second-order roots of Hicks (Table 2-8) and Speight G-28 (Table 2-9) tobacco seedlings after 14 days of growth in raw or

autoclaved soil. The 95% confidence [illegible] were estimated for these expected values.

The average numbers and total lengths of first-order and second-order roots per tobacco seedling in the two root infection trials fell variably within the range of expected values for various treatment combinations. Virtually all observed values of the root growth parameters for healthy or infected Speight G-28 tobacco seedlings (Tables 2-5 and 2-6) in raw or autoclaved soil fell within the ranges of expected values (Table 2-9). Root system development of healthy or infected Hicks tobacco seedlings in raw and autoclaved soils was inconsistent over trials. Very often observed numbers (Table 2-5) and total lengths (Table 2-6) of first-order and second-order roots per seedling of this cultivar in both soils fell below the minimum values expected (Table2-9). Patterns of deviation of these values from expected ranges were not obvious, but they were not related to infection of plants by P. parasitica var. nicotianae.

After 14 days of growth in soil infested with chlamydospores of the pathogen, up to 20% of observed infections per root system of tobacco occurred on second-order, third-order, or fourth-order roots. It was not clear whether these elements had become infected after tissues had matured, or if these root elements had become infected when they belonged to the first-order root class and tissues were just developing. The patterns of

infections observed after inoculation of various root tissues with zoospores suggested the latter to be the cause. Over 70% of the root tips inoculated with an average of 16 zoospores of P. parasitica var. nicotianae became infected (Table 2-10). Percentages of infection of older root tissues after inoculation with zoospores were significantly less as determined by Tukey's multiple comparison procedure for honestly significant differences ($p=0.05$). The growth of roots after inoculations at the root tip was variable. Very often infected roots continued to extend in length through 48 hours of incubation without becoming necrotic. In some instances, however, growth of infected roots ceased and the apical regions became necrotic. The pathogen was isolated readily from both types of roots. Roots inoculated at points distal to the root tip never developed such necrosis after infection. Direct microscopic observations of root tips 48 hours after inoculation revealed that in most cases sporangia had formed on root surfaces. Such secondary inoculum formation was not noted in association with inoculation points elsewhere on root surfaces.

Figure 2-1. Schematic representation of A) intact root system and B) dissected root system as described by the classification scheme of the morphometric root analysis system; 1,2,3 = root orders.

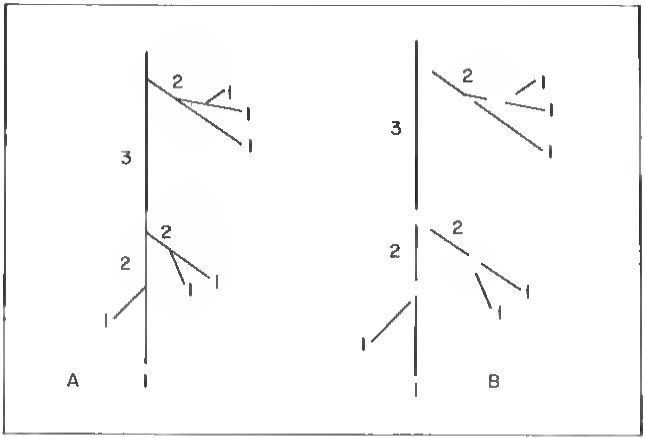

3
2
1
1
2
2
1
1
A
3
2
1
1
2
2
1
1
B

Figure 2-2. Average numbers of first-order (○—○), second-order (△— —△), and third-order (□— —□) roots observed per Hicks tobacco seedling during 15 days of growth in autoclaved field soil in the plant growth room.

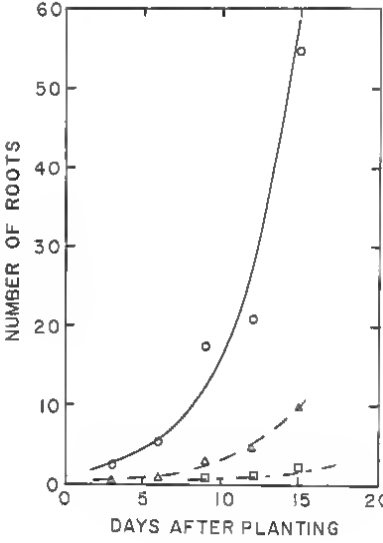

NUMBER OF ROOTS
60
50
40
30
20
10
0
0
5
10
15
20
DAYS AFTER PLANTING

TABLE 2-1. The numbers of roots and rates of root multiplication [illegible] Speight G-28 cultivars of tobacco after 15 days of plant growth [illegible] autoclaved field soil in the plant growth room

Cultivar	Soil	Root Order[a]	Number of Roots	$\bar{R}MR$ (no./no./day)[b]
Hicks	Autoclaved	1	55.7[c]	0.24
		2	10.2	0.23
		3	2.1	[illegible]
		4	0.2	—
	Raw	1	52.9	0.28
		2	9.5	0.23
		3	1.2	—
		4	0.2	—

TABLE 2-1. Continued

Caltivar	Soil	Root Order[a]	Number of Roots	RMR (no./no./day)[b]
Speight G-28	Autoclaved	1	50.8[c]	0.27
		2	8.5	0.70
		3	1.4	[illegible]
		4	0.4	--
	Raw	1	36.8	0.25
		2	5.9	0.20
		3	1.1	--
		4	0.2	--

[a] Root orders are as defined in the morphometric root analysis system (21)
[b] RMR = 1/N(dN/dt), relative multiplication rate of elements per root order; N = average number of elements per root order, t = time in days
[c] Values are the averages of numbers of elements per root order of at least eight seedlings.
[d] -- = Insufficient data to calculate rates.

TABLE 2-2. The total lengths of roots and rates of root extension for Hicks and Speight G-28 cultivars of tobacco after 15 days of growth in raw or autoclaved field soil in the plant growth room

Cultivar	Soil	Root Order[a]	Total Length (cm)	RER (cm/cm/day)[b]	Apparent UER (cm/root/day)[c]
Hicks	Autoclaved	1	121.5[d]	0.32	0.70
		2	19.3	0.30	[illegible]
		3	1.6	--[e]	--
		4	0.4	--	--
		All Roots	143.1	0.32	
	Raw	1	131.1	0.38	[illegible]
		2	18.7	0.35	[illegible]
		3	2.8	--	--
		4	0.2	--	
		All Roots	152.8	0.38	

TABLE 2-2. Continued

Cultivar	Soil	Root Order[a]	Total Length (cm)	RER (cm/cm/day)[b]	Apparent URR (cm/root/day)[c]
Speight G-28	Autoclaved	1	91.8[d]	0.34	0.65
		2	15.8	0.35	0.67
		3	2.2	--[e]	-
		4	0.5		
		All Roots	110.3	0.35	
	Raw	1	89.7	0.33	0.73
		2	12.4	0.42	0.97
		3	2.1		
		4	0.1	-	
		All Roots	104.3	0.34	

[a] Root orders are as defined in the morphometric root analysis system (21).

[b] RER = 1/L(dL/dt), the relative extension rate of elements per root order or per seedling; L = average total length of elements per root order or per seedling, t = time in days.

[c] URR = 1/N(dL/dt), the apparent unit extension rate or rate of extension per element of each order, N = average number of elements per root order.

[d] Values are the average of total lengths of all elements per root order of at least seedlings.

[e] -- = Insufficient data to calculate rates.

TABLE 2-3. The average lengths of elements within root orders of Hicks and Speight G-28 cultivars of tobacco during 15 days of plant growth in raw or autoclaved field soil in the plant growth room

Cultivar	Soil	Day	Mean Root Length (cm)/ Root Order[a] 1	2	3	4
Hicks	Autoclaved	3	0.9[b]	0.5	0.0	0.0
		6	1.5	1.0	0.0	0.0
		9	1.4	1.5	0.4	0.0
		12	2.0	1.2	1.3	0.0
		15	2.2	1.9	1.0	0.5
	Raw	3	1.0	0.1	0.0	0.0
		6	1.1	1.3	0.1	0.0
		9	1.5	1.7	0.4	0.0
		12	1.6	1.8	1.1	0.0
		15	2.5	2.0	1.4	0.0

TABLE 2-3. (cont.)

Cultivar	Soil	Day	Mean Root Length (cm) Root Order[a] 1	2	3	4
Speight G-28	Autoclaved	3	0.9[b]	0.1	[illegible]	[illegible]
		6	1.2	[illegible]	[illegible]	[illegible]
		9	1.5	[illegible]	[illegible]	[illegible]
		12	1.6	[illegible]	[illegible]	[illegible]
		15	1.9	[illegible]	[illegible]	[illegible]
	Raw	3	0.5	[illegible]	[illegible]	[illegible]
		6	1.3	[illegible]	[illegible]	[illegible]
		9	1.3	[illegible]	[illegible]	[illegible]
		12	1.9	[illegible]	[illegible]	[illegible]
		15	2.2	[illegible]	[illegible]	[illegible]

[a] Root orders are as defined in the morphometric analysis system (21).

[b] Values are the averages of mean root lengths [illegible] at least eight seedlings

Table 2-4. Observed infections on roots of two tobacco cultivars by Phytophthora parasitica var. nicotianae and inoculum efficiency after 14 days of plant growth in raw or autoclaved field soil infested with 50 chlamydospores of the pathogen per gram of soil

Trial	Cultivar	Soil	Infected Roots[a] Root Order 1[b]	2	3	4	Seedling	Inoculum Efficiency[c]
1	Hicks	Autoclaved	8.1	1.6	0.4	0.0	10.1	0.00
		Raw	10.6	2.2	0.5	0.0	13.3	0.004
	Speight G-28	Autoclaved	13.3	2.5	0.2	0.1	16.1	0.005
		Raw	8.5	2.1	0.3	0.0	10.9	0.003
2	Hicks	Autoclaved	9.4	2.3	0.5	0.1	12.3	0.004
		Raw	6.3	1.4	0.3	0.0	8.0	0.002
	Speight G-28	Autoclaved	4.5	0.8	0.1	0.0	5.4	0.002
		Raw	5.8	1.5	0.2	0.0	7.5	0.002

[a] Numbers of infected roots per root order or individual seedling were determined as the average of up to 15 asymptomatic, infected root systems which had been dissected completely by root order and plated onto selective medium (38).

[b] Root orders are as defined in the morphometric root analysis system (21).

[c] Efficiencies were determined from the average of the ratios of number of infected roots per seedling to total number of chlamydospores within the volume of soil containing each seedling.

Table 2-5. The relationship between infection of Hicks and Speight G-28 cultivars of tobacco by Phytophthora parasitica var. nicotianae and numbers of roots per seedling after 14 days of plant growth in raw or autoclaved field soil artificially infested with propagules of the pathogen

Trial	Cultivar	Soil	Chlamydospores/ g Soil	Number of Roots/ Root Order			
				1[a]	2	3	4
1	Hicks	Autoclaved	0	41.8x[b,c]	6.5x	1.3	0.3
			50	24.5x	4.0x	1.0	0.0
		Raw	0	30.8x	5.2x	1.0	0.0
			50	28.2x	4.8x	1.0	0.0
	Speight G-28	Autoclaved	0	45.0x	6.5x	1.3	0.3
			50	30.0x	5.5x	1.0	0.0
		Raw	0	34.8x	4.7x	1.5	0.5
			50	31.0x	6.0x	1.2	0.2

Table 2-5. Continued

Trial	Cultivar	Soil	Chlamydospores/ g Soil	Number of Roots/ Root Order			
				1[a]	2	3	4
2	Hicks	Autoclaved	0	28.7y[b,c]	3.7y	1.1	0.1
			50	28.0x	5.5x	1.1	0.1
		Raw	0	18.7y	3.0y	1.1	0.0
			50	26.0x	3.2x	1.2	0.2
	Speight G-28	Autoclaved	0	36.8x	5.2x	1.2	0.2
			50	27.8x	4.0x	1.2	0.2
		Raw	0	29.2x	4.2x	1.0	0.0
			50	31.2x	4.5x	1.3	0.3

[a] Root orders are as defined in the morphometric root analysis system (21).
[b] Values are the averages of numbers of elements per root order of six seedlings; within infested soil, averages are for infected root systems only.
[c] Values within corresponding root orders and treatment combinations of trials 1 and 2 which are followed by the same letter did not differ significantly. Comparisons of values for third-order and fourth-order roots were not made.

Table 2-6. The relationship between infection of Hicks and Speight G-28 cultivars of tobacco by Phytophthora parasitica var. nicotianae and total lengths of roots after 14 days of plant growth in raw or autoclaved field soil artificially infested with propagules of the pathogen

Trial	Cultivar	Soil	Chlamydospores/g Soil	Total Length (cm)				
				Root Order				
				1[a]	2	3	4	Seedling
1	Hicks	Autoclaved	0	94.4x[b,c]	11.5x	1.5	0.4	107.8
			50	53.1x	7.7x	1.3	0.0	62.1
		Raw	0	65.9x	10.9x	1.6	0.0	77.7
			50	65.2x	10.9x	1.6	0.0	[illegible]
	Speight G-28	Autoclaved	0	94.2x	13.2x	1.5	0.4	[illegible]
			50	58.8x	12.8x	1.0	0.0	72.6
		Raw	0	93.8x	11.0x	1.2	0.5	106.5
			50	63.7x	10.8x	1.7	0.3	76.5

Table 2 6. Continued

Trial	Cultivar	Soil	Chlamydospores/ g Soil	Total Length (cm) Root Order 1[a]	2	3	4	Seedlin
2	Hicks	Antoclaved	0	58.0y[b,c]	7.9x	1.1	0.2	[illegible]
			50	68.0y	10.3x	1.5	0.3	[illegible]
		Raw	0	40.4y	8.2x	0.6	0.0	[illegible]
			50	62.1x	7.8x	1.0	0.1	[illegible]
	Speight G-28	Autoclaved	0	75.9y	8.8x	1.4	0.1	[illegible]
			50	57.7x	8.5x	1.5	0.1	67.[illegible]
		Raw	0	57.3y	9.6x	0.8	0.0	67.7
			50	88.6x	11.3x	1.0	0.2	81.1

[a] Root orders are as defined in the morphometric root analysis system (21).
[b] Values are the averages of total lengths of elements per root order of six seedlin[illegible] within infested soil, averages are for infected root systems only.
[c] Values within corresponding root orders and treatment combinations of trials 1 [illegible] which are followed by the same letter did not differ significantly. Compari[illegible] values for third-order and fourth-order roots were not made.

TABLE 2 7. The relationship between infection of Hicks and Speight G-28 cultivars of tobacco by Phytophthora parasitica var. nicotianae and the average lengths of roots after 14 days of plant growth in raw or autoclaved soil artificially infested with propagules of the pathogen

Trial	Cultivar	Soil	Chlamydospores/ g Soil	Mean Root Length (cm) Root Order			
				1[a]	2	3	4
1	Hicks	Autoclaved	0	2.3[b]	1.7	1.2	0.4
			50	2.1	1.9	1.3	0.0
		Raw	0	2.2	2.7	1.9	0.8
			50	2.4	2.1	1.1	0.1
	Speight G-28	Autoclaved	0	2.1	2.1	1.1	0.8
			50	2.1	2.1	1.8	0.1
		Raw	0	2.1	2.3	1.8	0.1
			50	2.1	1.8	1.8	0.1

[illegible]

Trial	Cultivar	Soil	Chlamydospores/ g Soil	Mean Root Length (mm) Root Order			
				1[a]	2	3	4
2	Hicks	Autoclaved	0	2.0[b]	2.1	0.9	0.1
			50	2.5	1.9	1.4	0.3
		Raw	0	2.2	2.1	0.6	0.0
			50	2.5	2.6	1.0	0.1
	Speight G-28	Autoclaved	0	2.1	1.8	1.2	0.0
			50	2.1	2.3	1.2	[illegible]
		Raw	0	[illegible]	[illegible]	[illegible]	[illegible]
			50	[illegible]	[illegible]	[illegible]	[illegible]

[a]Root orders are as defined in the morphometric root analysis system [illegible].
[b]Values are the averages of mean lengths of elements per root order [illegible] within infested soil, averages are for infected root systems only.

TABLE 2-3. Expected ranges of values of numbers and total lengths of elements within root orders of Hicks cultivar of tobacco after 14 days of plant growth in raw or autoclaved field soil in the plant growth room

Soil	Root Order[a]	Number of Roots			Total Length (cm)		
		min.[b]	mean	max.	min.	mean	max
Autoclaved	1	34.1	43.3	55.1	79.8	103.5	134.7
	2	6.7	8.3	10.2	8.4	13.3	21.1
	All Orders[c]				89.1	112.2	141.2
Raw	1	38.5	46.1	55.1	72.2	96.5	129.0
	2	5.3	6.3	7.5	8.1	14.0	24.3
	All Orders				75.9	108.9	156.3

[a] Root orders are as defined in the morphometric root analysis system (21).
[b] Minimum and maximum values are the limits of the 95% confidence intervals about means; the mean values were derived from interpolations of the linear regressions of root growth parameters on time.
[c] Includes all root orders per seedling.

TABLE 2-9. Expected ranges of values of numbers and total lengths of elements within root orders of Speight G-28 cultivar of tobacco after 14 days of plant growth in raw or autoclaved field soil in the plant growth room

Soil	Root Order[a]	Number of Roots			Total Length (cm)		
		min.[b]	mean	max.	min.	mean	max
Autoclaved	1	27.9	40.0	87.4	48.9	74.4	113.9
	2	4.4	6.2	8.7	4.2	13.1	40.4
	All Orders[c]				57.4	83.1	120.3
Raw	1	18.5	32.5	56.8	39.6	77.5	151.4
	2	2.4	4.9	9.7	8.3	14.3	24.5
	All Orders				47.5	91.8	177.7

[a] Root orders are as defined in the morphometric root analysis system (21).
[b] Minimum and maximum values are the limits of the 95% confidence intervals about means; the mean values were derived from interpolations of the linear regressions of root growth parameters on time.
[c] Includes all root orders per seedling.

TABLE 2-10. The relationship between the point of inoculation with zoospores of *Phytophthora parasitica* var *nicotianae* and infection of roots of Hicks cultivar tobacco

Inoculation Point[a]	Percentage of Roots Infected[b]
Root Tip	73.3a[d]
2 cm Behind Tip[c]	10.0b
Second-Order Root	4.3b

[a] A microdrop containing an average of 16 zoospores was applied at each inoculation point.

[b] Values are the means of percentages of infection from three inoculation trials; in each trial percentages of infection were based on ten replicate point inoculations.

[c] Inoculation points were located on the surfaces of first-order roots.

[d] Values with different letters were significantly different as determined by Tukey's multiple comparison procedure for honestly significant differences ($p=0.05$).

Discussion

Descriptions of root system development traditionally have been based on a developmental model (4). In that scheme roots are defined by their order of appearance. Roots produced directly from the base of a shoot are defined as axes. Lateral roots emerging from the axes are referred to as primary laterals, elements arising from these roots are termed secondary laterals and so forth. According to the model, the full length of any particular root belongs to the same lateral group.

The use of the developmental model for analyses of patterns of infections by pathogens on individual root systems is rather cumbersome and may lead to misinterpretations of these patterns. In particular it may be difficult to quantify points of infection in relation to susceptibilities of root tissues because all tissues of a root, regardless of physiological age, are placed within the same category.

The morphometric root analysis system devised by Fitter (21) offers a more definitive model for such evaluations. Within the scheme of this system, root systems are divided into regions of increasing tissue maturity which correspond to increasing root order. Root tissues which have just formed belong initially to the first-order root class. As these segments of tissues mature, and further branching occurs proximal to the apical

[illegible] basic segments become part of successively higher root orders. Changes in the relative proportions of [illegible] root systems which are of various physiological [illegible] are reflected in the changes in numbers and lengths of [illegible] within various root orders.

Although branching of root systems is defined according to different orientations in the developmental and morphometric models, similar patterns of root system development are described by the two models. In particular, increases in numbers and total lengths of elements in each lateral group or root order have been shown to proceed exponentially during early plant growth (21, 58, 74). Expressions describing relative rates of such increases have been derived analogously in the two models in reference to the different units of classification (21, 58, 74).

Relatively few detailed quantitative descriptions of root system development have been provided using either of these models. The developmental model has been used most often to describe the development of root systems of various field crops grown under different fertilization regimes (28, 58, 59, 72, 87). Bloomberg (7) has utilized this model to quantify root system development of Douglas-fir seedlings. The morphometric analysis system has been utilized to describe in detail root growth of two herbaceous plant species, Poa annua and Rumex crispus (21).

In the present study initial descriptions of the development of tobacco root systems have been provided. After 15 days of growth in raw or autoclaved soil, the total lengths of root systems of Hicks and Speight G-28 tobacco did not differ significantly. During this period of plant growth, rates of root multiplication and extension varied somewhat in association with various combinations of cultivar and soil treatment. The lack of differences in total seedling root lengths at the end of the growth period suggested that both rate of root multiplication and rate of extension were important in determining ultimate seedling root length. It appears also that a greater value of one of these rates may have compensated for a lesser value of the other to produce equivalent total lengths of seedling roots at the end of 15 days of growth. For example, although the rate of multiplication of first-order roots of Speight G-28 tobacco was greater in autoclaved soil as compared to raw soil, the apparent unit extension rate of first-order roots of this cultivar was greater in raw soil as compared to autoclaved soil (Tables 2-1 and 2-2). Both combinations of rates gave rise to equivalent average total seedling root lengths. The nature of such compensating effects are not known. Differences in these rates may play an important role in defining the development of individual components of seedling root systems.

Although the total lengths of root systems of the two cultivars were not significantly different after 15 days of growth, total lengths of Hicks tobacco tended to be greater than total lengths of Speight G-28 tobacco. It may be that significant differences would have become apparent given sufficient additional time or additional repetitions of the test. An extension of the period of root growth may be necessary to evaluate the influences of small differences in rates of root multiplication and extension on root system development.

The values of parameters of root growth estimated for tobacco in these trials must be accepted only for the conditions of these trials. Parameter estimates may depend on a number of environmental and cultural variables within any particular experiment. For example, direct comparisons of absolute values of rates of root multiplication and extension for tobacco cannot be made with those estimated by Fitter (21) for P. annua and R. crispus because the latter plant species were begun from germinated seed and were followed through 41 days of growth. In contrast, analyses of tobacco root growth were begun at the time of transplant of 14-day-old seedlings. At that age tobacco seedlings all had a single first-order root whereas seedlings of the two species examined by Fitter (21) had produced approximately 20 to 120 first-order roots. Additionally, tobacco plants were grown in a plant growth room and were not fertilized after transplant; plants in

Fifteen (21) experiments were fertilized regularly and were grown in a greenhouse.

Evaluations of the development of root systems of tobacco in relation to a short time provided only a partial description of root system formation; still missing are quantitative descriptions of root growth in relation to space over the entire crop production period. Such descriptions are of importance in comprehending the relationship between root density and inoculum distribution both vertically and horizontally in the soil profile. Bloomberg (7, 8) evaluated this relationship between root system development of Douglas-fir seedlings by soil depth and inoculum density of F. oxysporum. He was able to incorporate estimates of root system development into a predictive model for damping-off and root rot of seedlings caused by this pathogen. Dryden and van Alfen (15) and Hancock (30) also have evaluated such relationships between root system development and inoculum densities of pathogens of pinto beans and alfalfa, respectively. In particular, Dryden and van Alfen (15) were able to demonstrate the relationships of root infections to time and increasing depth in the soil profile. Within these latter investigations infections were quantified either in relation to the proportion of infected rootlets (30) or to a unit length of total roots (15). Such units of quantification did not define root system morphology sufficiently to provide insight into the development of

epidemics involving soilborne pathogens on [illegible] systems. For example, neither of these units of quantification allowed detailed evaluations of early infections of root systems in relation to the development of susceptible root tissues.

Processes involved in the development of epidemics associated with soilborne pathogens generally have been evaluated through quantification of disease in terms of incidence on a whole plant basis. Disease incidence as a measure of disease progression represents the end result of numerous cycles of interactions between populations of a host plant and pathogen. Typically such incidence values have been transformed on the basis of mathematical models (2, 3, 37, 75, 91) to provide biological interpretations of the processes of disease development. Unfortunately, models have been based on contentious assumptions and interpretations of processes involved in disease development have come under challenge. To reduce the complications of such interpretations, the investigations of early root infection of tobacco by P. parasitica var. nicotianae were established to measure more directly the relationship of inoculum and susceptibilities of root tissues to infection.

An imposed short period of growth of tobacco in soil infested with this pathogen allowed for quantification of early interactions between roots of these plants and P. parasitica var. nicotianae. An inoculum density much

greater than that typically found as initial inoculum in the field was used in the present experiments, however, this density did not appear to overwhelm the system in the 2 weeks of testing. The large proportions of asymptomatic plants and the low average numbers of observed infected roots per infected seedling supported this contention. The variation in numbers of infections observed per infected seedling supported as well the strongly stochastic nature of the infection process in situ. Such variations in numbers of infections would be expected at early stages of any epidemic.

Although the numbers of observed infected roots per infected seedling varied considerably within and between trials, differences rarely were significant. Numbers of infected roots per infected seedling and inoculum efficiency did not appear to be sufficiently sensitive to serve as criteria to compare influences of cultivar or soil ecosystem on early infection events. It was thought that inoculum efficiency, in particular, would provide a useful criterion for such evaluations because it is dependent on both disease incidence and numbers of infections per infected seedling. Further reductions in inoculum density may be necessary to increase the sensitivities of these parameters in such short term studies.

The values of inoculum efficiency in this trial were very low. Such low values reflect the low probability of compatible interactions between susceptible root tissues

end pathogen propagules in soil during only 2 weeks of tobacco growth. Efficiency would be expected to increase with increasing time of plant growth in infested soil. The low values also may have been an artifact associated with estimations derived from the ratios of numbers of infected roots to numbers of pathogen propagules. It was not possible to determine if more than one infection had occurred per infected root. If such an occurrence was common, then the true values of inoculum efficiency would have been greater than those observed in these trials.

Estimates of efficiency of inoculum of soilborne pathogens have been provided in only one other pathosystem. Tomimatsu and Griffin (89) reported the efficiency of microsclerotia of Cylindrocladium crotalariae for infection of peanuts at 103%. This value, however, was estimated on the basis of numbers of infections per germinated sclerotium placed within the region of the root surfaces of peanut plants. Only 0.27 to 0.28% of these observed infections resulted in necroses of roots.

The lack of differences in observed numbers of infected roots per infected tobacco plant of cultivars variably resistant to P. parasitica var. nicotianae indicates that resistance is expressed at stages of disease development beyond initial infection. Several authors have reported such a lack of differential response of susceptible and resistant plant cultivars to initial infections by Phytophthora spp. (6, 10, 26, 27, 60, 65)

Resistance instead was reported in such studies to be expressed through reductions in the rates and extensiveness of progressive root tissue colonization by pathogens. In most of these trials, however, the susceptibilities of cultivars to infection were compared by immersing root tips or root systems into concentrated suspensions of zoospores. Mechanisms of resistance to initial infection may have been overwhelmed by the high numbers of zoospores which encysted and infected within a limited region behind root tips. Within soil such large numbers of zoospores are not likely to be available for infection.

Although the accumulation of zoospores of Phytophthora spp. behind root tips and subsequent infection was demonstrated in the above trials, no information previously was available as regards the relative susceptibilities of various root tissues of tobacco to infection by P. parasitica var. nicotianae. The results of the present point inoculation trials suggested a limited region of high susceptibility to infection behind apical meristems of first-order roots. The full extent of these zones was not revealed although it must have been less than 2 cm in length. The low percentage of successful infection of second-order roots suggested that infections observed on higher-order roots in these short term, growth-room trials occurred when tissues were just developing as components of the first-order class

A restricted region of susceptibility to infection associated with tissues just behind apical meristems was noted as well in a strawberry cultivar susceptible to infection by P. fragariae (26). Although zoospores of the pathogen were found to aggregate and encyst on surfaces of strawberry roots as far as 4 cm behind apical meristems, infection did not occur beyond 0.6 to 0.7 cm from the root tip.

The possibility also exists for regions of increased susceptibility on older root tissues in association with wounds. Such an influence on susceptibility has been reported in relation to infection of roots of shortleaf pine by P. cinnamomi and tobacco by P. parasitica var. nicotianae (16, 56). The importance of wounds in increasing opportunities for infection by soilborne pathogens likely would increase with time of growth in field situations.

The continuation of root length extension observed in some cases after inoculation of root tips with P. parasitica var. nicotianae provided insight into the lack of differences in patterns of root branching of healthy and infected seedlings observed after 14 days of tobacco growth. It seems likely that, during that period of plant growth, root extension and branching continued after infection by the pathogen. With sufficient additional time, root necrosis likely would have occurred and patterns of root growth of healthy and diseased plants perhaps would

[illegible] diffusion. These experiments could not be continued to such a period of time because the extensiveness of root growth at such a time would preclude limited analysis of complete root systems to very few plants. The results of these short term trials truly may have reflected events which occurred early in the process of plant infection.

Controlled root growth of tobacco was achieved variably in two trials. Growth of Speight G-28 tobacco was controlled well enough that numbers and lengths of roots fell within expected ranges during the 14 days of plant growth in infested soils. Root growth of Hicks tobacco was controlled less effectively. The degree of control attained, however, was encouraging when considering the variations that could be expected from utilizing transplants. Variations in patterns of root growth might be less in trials in which plants were begun from seed which had been screened for uniformity. Increased control of plant growth is certainly desirable in evaluations of the contributions of the host root component to pathosystem behavior.

CHAPTER III
THE DEVELOPMENT OF MICROBIAL COMMUNITIES [illegible]
TOBACCO ROOT SYSTEMS

Introduction

Manipulations of introduced microbial antagonists to control soilborne diseases effectively are dependent upon the maximization of opportunities for interactions between populations of a pathogen and antagonists. A thorough understanding of the biological activities of a pathogen and antagonists within a soil ecosystem must be developed if adequate opportunities for efficient interactions between these populations are to be provided. This concept is of great importance in developing strategies for biological control of black shank of tobacco (Nicotiana tabacum L.), which is incited by the soilborne pathogen, Phytophthora parasitica Dast. var. nicotianae (Breda De Haan) Tucker.

Initial populations of this pathogen in soil are extremely low and highly aggregated (20, 35). In the absence of host roots, propagules of this pathogen within soil are predominently thick-walled chlamydospores. Attempts to reduce low initial populations of P. parasitica var. nicotianae even further by encouraging interactions with populations of antagonists would be futile economically. Efficient contact between any two or more

microbial populations within non-rhizosphere soil is [illegible] likely to be attained because of the extensiveness and heterogeneity of the environment in which these populations function (1). A more appropriate region in which to manipulate antagonists would be the rhizosphere of the tobacco plant. It is within this region that the pathogen is biologically active and susceptible to influence by antagonists. In addition, this region represents a much reduced volume of soil with which to be concerned.

Control of black shank by the establishment of a microbial community antagonistic to P. parasitica var. nicotianae within the rhizosphere of tobacco plants has not been reported. A prerequisite to the development of a community antagonistic to the pathogen within the rhizosphere of tobacco is an understanding of the population dynamics and patterns of soil and root surface colonization by various microorganisms within this region. The present investigations were designed to examine the patterns of development of microbial communities associated with tobacco root systems in both stable and unstable soil ecosystems over time. Spatial patterns of fungal colonization of root surfaces also were examined in these two ecosystems.

Materials and Methods

Microbial communities associated with roots [illegible] were evaluated periodically during plant growth [illegible] soil (Blichton sand) collected from Gainesville, [illegible] Soil was air-dried and passed through a 1-mm sieve prior to use. One half of the soil was autoclaved for 1 hour on each of two successive days, the remaining raw soil was not treated.

Eighty grams of raw or autoclaved field soil were layered over 15 g of autoclaved builder's sand in 100-ml, polypropylene beakers. A single, 2-week-old seedling of the tobacco cultivar Hicks was transplanted into each beaker. Seedlings were grown in a glass greenhouse for 28 days at 16 to 30 C. Plants were watered from above on alternate days.

Every 7 days 10 seedlings were removed from both raw and autoclaved soils. Whole root systems were teased from soil using forceps and excess soil was removed from root surfaces by gentle shaking. Soil still adhering to roots was considered to be part of the rhizosphere. Rhizosphere soil was removed from the 10 bulked root systems removed from each type of soil by swirling roots in 50 ml of sterile deionized water for 1 min. Root systems then were removed for further processing.

Rhizosphere soil suspensions were diluted appropriately at each sampling date and 1-ml samples of suspensions were pipetted onto media selective for fungi, bacteria, and actinomycetes. Estimates of population densities were made from average numbers of colonies developing on 10 plates per medium. Populations of general fungi were determined from soil suspensions pipetted into Petri plates containing molten potato dextrose agar amended with 50 mg of chlortetracycline hydrochloride (50% a.i., Sigma Chemical Co., St. Louis, MO 06817) and 1 ml of Tergitol NP-10 (Union Carbide Corp., Danbury, CT 06817) per liter of medium. Plates were incubated at 25 C under 12 hours of light (300 μEin/m^2/sec) per day and examined at 7 - 10 days for colony formation. Populations of Pythium spp. were determined from soil suspensions pipetted onto the surface of the solidified selective medium of Kannwischer and Mitchell (40) as described in Chapter II. Colonies were counted after the plates had been maintained in the dark at 25 C for 48 hours.

Populations of general bacteria and actinomycetes were determined from soil suspensions pipetted into Petri plates containing molten, one-tenth strength tryptic soy agar (Difco Laboratories, Detroit, MI) amended with 50 mg cycloheximide (Sigma Chemical Co.) per liter of medium. Plates were examined for colonies of general bacteria and actinomycetes after 2 weeks of incubation in the dark at 25 C. Populations of fluorescent Pseudomonas spp. were

determined from soil suspensions pipetted [illegible] containing molten, modified King's medium B (77 [illegible] medium contained 20 g proteose peptone no 3 (Difco Laboratories), 1.5 g anhydrous K_2HPO_4, 1.5 g $MgSO_4 \cdot 7H_2O$, 10 ml glycerol, 20 g Difco Bactoagar (Difco Laboratories), 75 mg cycloheximide, and 45 mg novobiocin (sodium salt, Sigma Chemical Co.) per 1.0 liter of deionized water. The medium was modified further by the replacement of penicillin G with 50 mg of ampicillin (sodium salt, Sigma Chemical Co.) per liter of medium. After incubation in the dark for 4 days at 25 C, plates were examined under ultra-violet light for colonies producing diffusible fluorescent pigments.

Estimates of population densities of microorganisms on surfaces of roots were made in a manner similar to that described by Rovira et al. (76). Bulked root systems devoid of rhizosphere soil were shaken for 30 min in 50-ml, sterile, deionized water blanks containing 5 g of 3-mm glass beads. Flask contents were passed aseptically through a 75-μ nylon screen, diluted appropriately, and plated on selective media as before.

Estimates of population densities of microorganisms in raw and autoclaved, non-rhizosphere soil were made at each sampling date from single samples taken to a depth of about 3 cm with a surface-disinfested cork borer. Samples were diluted appropriately and plated in a manner similar to that for rhizosphere and root surface samples. Total

population densities of the various microorganisms in the rhizosphere, root surface, and non-rhizosphere soil regions of raw and autoclaved soil were compared at each sampling date using Lohrding's test, which assumes equal coefficients of variation (25).

The dispersion of fungal hyphae on the surfaces of first-order roots of tobacco was determined by direct observation. At each sampling date three additional seedling root systems were removed from raw and autoclaved soil and rinsed gently to remove adhering rhizosphere soil. Each root system was vacuum-infiltrated for 3 min with 0.005% brilliant cresyl blue in phosphate buffer at pH 10.2. Each root system then was rinsed briefly in phosphate buffer (pH 7.4) to remove excess stain.

Randomly selected first-order roots from each seedling, as defined in the morphometric root analysis system (21) and described in Chapter II, were selected for evaluation. Five microscope fields were selected systematically along the full length of each first-order root. Within each field determinations were made of the number of intersects between fungal hyphae and grid lines of a Whipple disc. Estimates of hyphal length were made using Tennant's modified line intersect method (86). Between 2 and 20 randomly selected first-order roots were examined per root system depending on the age of seedlings. Frequencies of the numbers of hyphal intersects within all microscope fields per seedling were tabulated

[illegible] estimates of the dispersion parameter, k, associated with the negative binomial distribution were developed from analyses utilizing the computer program of Gates and Ethridge (24). Hyphal aggregation on root surfaces was estimated as a function of k using Lloyd's indices of mean crowding and patchiness (47). Isolations and root colonization trials were performed twice.

Results

Population densities of various microorganisms are presented as the averages of estimates of the two trials. Average population densities of total detectable fungi in rhizospheres of Hicks tobacco plants fluctuated considerably during 28 days of plant growth in raw and autoclaved soil (Fig. 3-1). Within autoclaved soil 315 x 10^2 propagules per gram of oven-dried, rhizosphere soil were associated with roots of plants grown for 7 days. Estimates of total populations within individual trials differed considerably and ranged from 156 x 10^2 to 474 x 10^2 propagules per gram of rhizosphere soil in trial 1 and trial 2, respectively. Average population densities declined rapidly and from day 14 onward remained less than 20 x 10^2 propagules per gram of soil. Minimum and maximum densities of 1 x 10^2 and 32 x 10^2 propagules

per gram of rhizosphere soil were detected. Average densities of fungi in the rhizospheres of plants grown in raw soil varied less over time, a maximum average density of 144 x 10^2 propagules per gram of soil was encountered at day 14. Population densities in the rhizosphere regions of the two soil ecosystems differed significantly only at day 14 ($p=0.10$). Densities at day 7 likely were not significantly different between the two ecosystems because of the large variation in estimates of densities in the autoclaved soil system over trials. Average population densities of total fungi within the rhizospheres of tobacco in the two soil ecosystems essentially were the same after day 21.

Average population densities of total fungi associated with root surfaces of Hicks tobacco plants fluctuated in a manner similar to that for populations in the rhizosphere (Fig. 3-2). A maximum average density of 2920 x 10^2 propagules per gram of oven-dried roots was associated with tobacco grown in autoclaved soil for 7 days. Estimates of densities within individual trials varied between 2155 x 10^2 and 3685 x 10^2 propagules per gram of oven-dried roots in trial 1 and trial 2, respectively. Densities of fungi associated with root surfaces in this soil environment declined steadily with further plant growth. Average population densities of fungi associated with root surfaces of plants grown in raw soil were fairly constant through 21 days of plant growth. Densities of fungi

associated with surfaces of tobacco roots in raw [illegible] autoclaved soil differed significantly only at day 7 ($p=0.10$).

Fungal propagules were removed efficiently from root surfaces using glass beads. Microscopic examination of root systems revealed no fungal hyphae on root surfaces after shaking with glass beads. Epidermal cells and root hairs did not appear to be disrupted. Preliminary root isolation trials utilizing this procedure had revealed that agitation for 30 min provided recovery of more than 90% of detectable fungal and bacterial propagules.

Population densities of total fungi were not significantly different in raw and autoclaved, non-rhizosphere soils (Fig.3-3). Densities of total fungi within raw soil increased steadily from day 7 through day 21 and declined thereafter. Within autoclaved, non-rhizosphere soil, average densities fluctuated between 1 and 4 x 10^2 propagules per gram of soil.

Population densities of bacteria in the rhizosphere (Fig. 3-4) and at root surfaces (Fig. 3-5) of plants grown in raw or autoclaved soil did not differ significantly ($p=0.10$). Minimum and maximum average densities detected in the rhizosphere were 1 x 10^7 and 16 x 10^7 colony forming units per gram of oven-dried soil, respectively. Minimum and maximum average densities detected at root surfaces were 3 x 10^7 and 47 x 10^7 colony forming units per gram of oven-dried roots, respectively. Densities also

were not significantly different in raw or autoclaved non-rhizosphere soil (Fig. 3-6) and average densities varied between a minimum and maximum of 1×10^7 and 10^7 colony forming units per gram of oven-dried soil, respectively.

Population densities of detectable actinomycetes in the rhizosphere (Fig. 3-4) or at root surfaces (Fig. 3-5) of plants grown in raw soil fluctuated around 10^6 colony forming units per gram of oven-dried soil or roots. Densities in raw, non-rhizosphere soil also fluctuated about this density (Fig. 3-6). Actinomycetes were not detectable in any region of the ecosystem with autoclaved soil during the first 14 days of tobacco growth, at days 21 and 28, however, densities of less than 2×10^5 colony forming units per gram of soil or roots were noted sporadically in all three regions. Average densities of actinomycetes within these regions of the ecosystem with autoclaved soil at days 21 and 28 always were significantly less than within corresponding regions of raw soil ($p=0.10$).

Average population densities of fluorescent Pseudomonas spp. within the rhizosphere (Fig. 3-7) and at root surface (Fig. 3-8) of tobacco, and within non-rhizosphere soil (Fig. 3-9) of the ecosystem with raw soil did not differ significantly ($p=0.10$) from densities in corresponding regions of the ecosystem with autoclaved soil during 28 days of tobacco growth. Average densities

of these bacteria in the rhizosphere and at the root surfaces of plants in both raw and autoclaved soil varied between 400 and 20,000 colony forming units per gram of soil or roots. No obvious patterns were observed in fluctuations of populations over time. Average population densities of fluorescent Pseudomonas spp. in raw, non-rhizosphere soil varied within a narrow range from 40 to 140 colony forming units per gram of soil. Average population densities in autoclaved soil increased from 6 to 2,100 colony forming units per gram of soil between days 7 and 28.

The numbers of fungal taxa encountered in each region of the two soil ecosystems were determined. Taxa included predominantly genera, families, and fewer defined species. The numbers of taxa encountered were always greater within the various regions of the ecosystem with raw soil than within corresponding regions of the ecosystem with autoclaved soil (Fig. 3-10). In raw soil maxima of 18, 18, and 16 taxa were encountered in the rhizosphere, root surface, and non-rhizosphere soil regions, respectively. The number of taxa encountered in each region of the ecosystem with autoclaved soil was always less than the number encountered in each corresponding region of raw soil. Fewer than five taxa were encountered initially in the rhizosphere and at the root surfaces of plants grown in autoclaved soil. By day 28 the numbers of taxa recovered from the rhizosphere and root surfaces increased to 10 and

11, respectivaly [illegible] non-rhizosphere soil nine fungal taxa were encountered at day 7 and little fluctuation was obaarved theraafter.

The structure of fungal communities within the ecosystem with raw soil contrasted sharply with the communities in the ecasystem with autoclaved soil. The compositions of communities of fungi within tha various regions of raw soil were fairly constant over time. Fungi of the genera Penicillium, Trichoderma, Aspergillus, Fusarium, and Cylindrocarpon colonized the rhizosphera (Table 3-1) and root surfaces (Table 3-2) rapidly. Throughout 28 days of tobacco growth, these fungi accounted for 75 and 60 percent of the total recaverable fungal propagulas associated with the rhizosphere and root surfaces, respectively. Less dominant genera and families often colonized root systems more slowly or sporadically durieg plant growth. With few exceptions fungi represanting genera and families encountered in free soil (Table 3-3) also colooized the rhizospheres or root surfaces of tobacco plants. Conversely, although Paatalotia sp. and Alternaria sp. were recovered from either the rhizosphare ar root surface regions of tobacco plants, they presumably occurred in non-rhizosphere soil at densities too low to be detected.

Structurea of communities within the ecosystem with autoclaved aall varied over time. Although population densities of total fungi in tha rhizosphere (Table 3-4) and

associated with root surfaces (Table 3-5) of tobacco plants were very high after 7 days of plant growth, over 95% of these populations were accounted for by fungi of three taxa, which included yeasts, Fusarium sp., and Cylindrocarpon sp. With time the dominance of these few taxonomic groups was diminished and a greater proportion of the total fungal population was comprised of other genera which included Penicillium, Trichoderma, and Cladosporium. The community of fungi associated with autoclaved, non-rhizosphere soil (Table 3-6) included a greater number of genera and families at earlier sampling dates than did the communities in the rhizosphere or root surface regions of this ecosystem (Fig. 3-10).

Fungal hyphae were observed commonly on root surfaces of plants grown in both soil ecosystems; no reproductive or resting structures were observed. Fungal colonization of the surfaces of first-order roots of tobacco plants was much more extensive within raw soil than within autoclaved soil. Approximately 70% of the microscope fields selected along the lengths of such roots from raw soil contained fungal hyphae, only 30% of such fields contained hyphae within autoclaved soil.

The average length of fungal hyphae along the length of first-order roots was greater in association with plants grown in raw soil as compared to plants grown in autoclaved soil (Fig. 3-11). The average length of hyphae along surfaces of first-order roots in autoclaved soil remained

fairly constant over time and varied between [illegible] cm of hyphae per 10 cm of first-order root length. The average length of hyphae along surfaces of first-order roots in raw soil increased rapidly from day 7 to day 14; thereafter average lengths fluctuated between 15.7 and 21.2 cm hyphae per 10 cm of first-order roots.

Fungal hyphae were dispersed along the surfaces of first-order roots of tobacco plants in an aggregated fashion. The negative binomial distribution described adequately over 90% of the populations of fungal hyphae sampled; the poisson distribution did not describe any of these populations. The values of the dispersion parameter, k, associated with fungal populations on surfaces of first-order roots of plants grown in raw or autoclaved soil were very low and varied between 0.05 and 0.85. Values of k were always less in association with fungal colonization of root surfaces in autoclaved soil than in raw soil.

The degree of aggregation of fungal hyphae on root surfaces was evaluated utilizing Lloyd's index of mean crowding and Lloyd's index of patchiness (47). Mean crowding estimated the relative crowding of hyphae in colonized regions along the length of root surfaces in terms of the average number, per hyphal intersect, of other hyphal intersects with reticule grid lines per microscope field in which hyphae were observed. The index ignored those microscope fields which were devoid of hyphae. This index is defined as a function of the mean number of hyphal intersects per microscope field, m, and k such that

$$\overset{*}{m} = m + m/k$$

[illegible] of hyphae in colonized regions increased, the value of mean crowding also increased. Mean crowding of fungal hyphae was greater in association with roots in raw soil than in autoclaved soil after 7 days of plant growth (Fig. 3-12). Values of mean crowding of hyphae on the surfaces of roots in the former soil ecosystem varied between 35 and 50 after an initial increase from day 7. Mean crowding of hyphae on surfaces of roots within the autoclaved soil ecosystem was fairly constant throughout 28 days of plant growth and was always less than 12.

The aggregation of colonized regions along surfaces of first-order roots was described by Lloyd's index of patchiness. This index is defined as the ratio of mean crowding to the mean number of hyphal intersects per microscope field and subsequently is related to the k parameter such that

$$LIP = \overset{*}{m}/m = 1 + 1/k.$$

Fig. 3-1. Populations of total detectable fungi in the rhizosphere of Hicks tobacco plants grown in raw (●——●) or autoclaved (○— —○) field soil for 28 days.

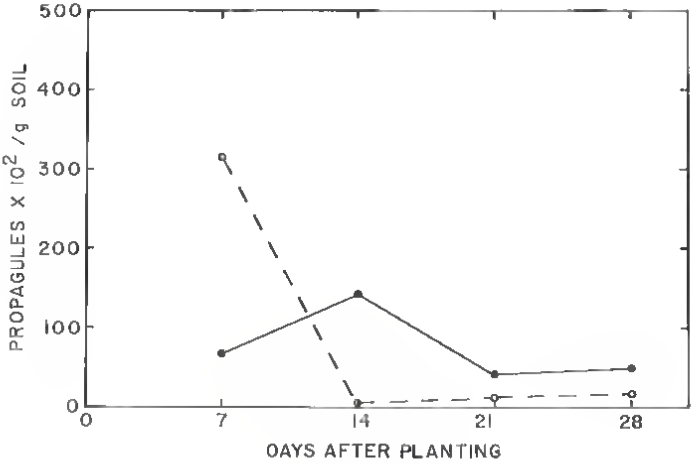

500
400
300
200
100
0
PROPAGULES X 10^2 / g SOIL
0
7
14
21
28
DAYS AFTER PLANTING

Fig. 3-2. Populations of total detectable fungi associated with root surfaces of Hicks tobacco plants grown in raw (●—●) or autoclaved (○— —○) field soil for 28 days.

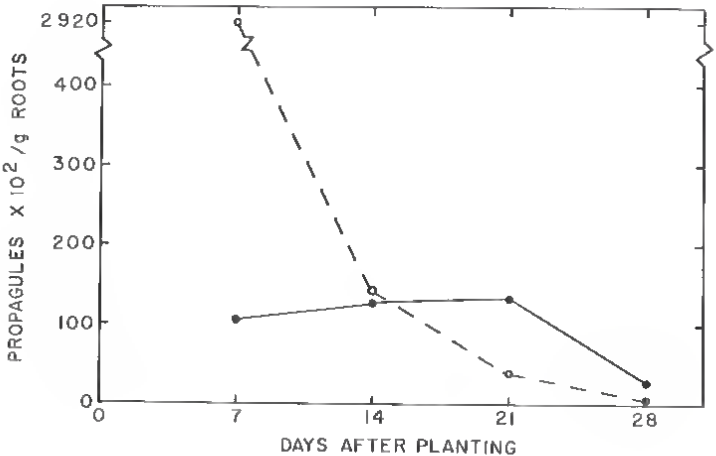
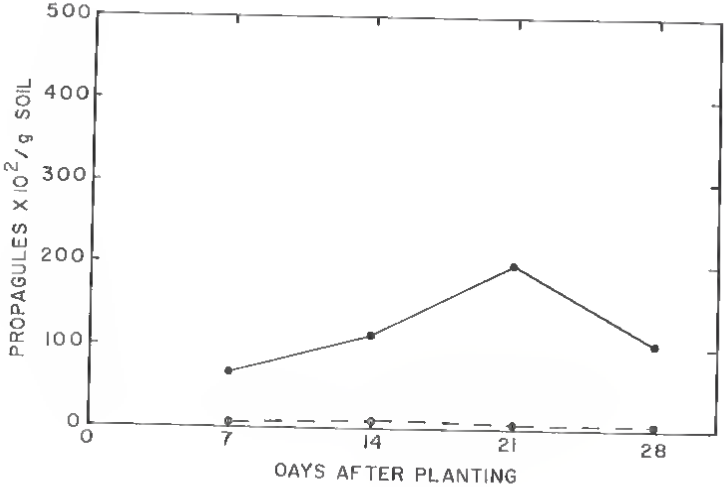

2920
400
300
200
100
0
PROPAGULES X 10² /g ROOTS
0
7
14
21
28
DAYS AFTER PLANTING

Fig 3-3. Populations of total detectable fungi in raw (●—●) or autoclaved (○—○) field soil during 28 days of growth of Hicks tobacco plants.


500
400
300
200
100
0
PROPAGULES X 10^2/g SOIL
0
7
14
21
28
DAYS AFTER PLANTING

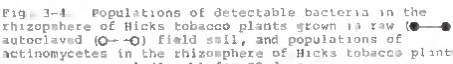


Fig. 3-4. Populations of detectable bacteria in the rhizosphere of Hicks tobacco plants grown in raw (●—●) or autoclaved (○—○) field soil, and populations of actinomycetes in the rhizosphere of Hicks tobacco plants grown in raw soil (Δ—Δ) for 28 days.

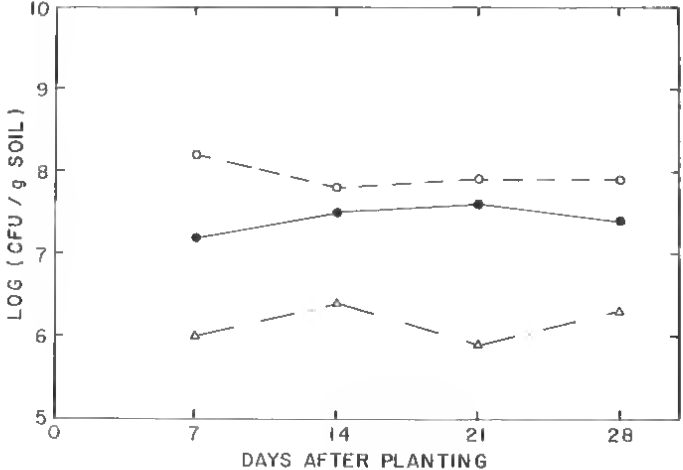

10
9
8
7
6
5
LOG (CFU / g SOIL)
0
7
14
21
28
DAYS AFTER PLANTING

Fig. 3-5. Populations of detectable bacteria associated wi
root surfaces of Hicks tobacco plants grown in raw (●—●) o
autoclaved (○—○) field soil, and populations of
actinomycetes associated with root surfaces of Hicks tobacc
plants grown in raw soil (△— △) for 28 days.

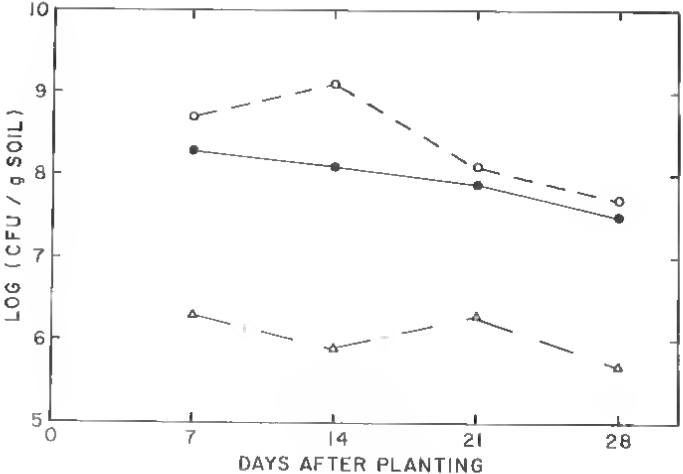
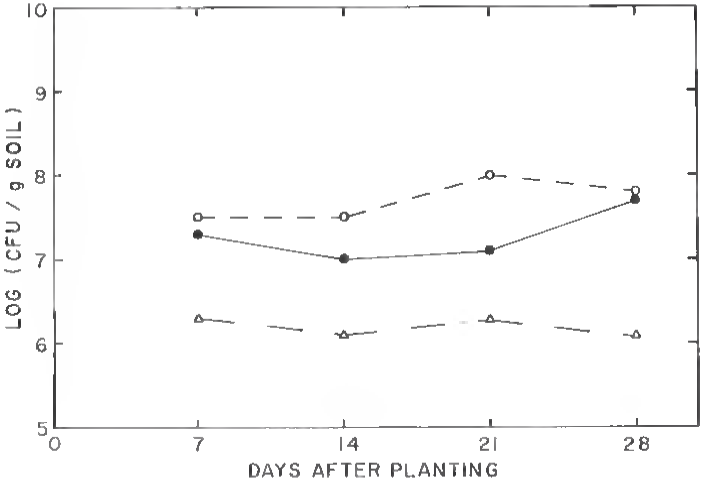

10
9
8
7
6
5
LOG (CFU / g SOIL)
0
7
14
21
28
DAYS AFTER PLANTING

Fig. 3-6. Populations of detectable bacteria (●——●) and actinomycetes (Δ— —Δ) in raw field soil, and populations of bacteria (O— —O) in autoclaved field soil during 28 days growth of Hicks tobacco plants.


10
9
8
7
6
5
LOG (CFU / g SOIL)
0
7
14
21
28
DAYS AFTER PLANTING

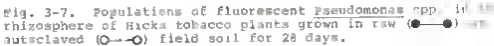


Fig. 3-7. Populations of fluorescent Pseudomonas spp. in the rhizosphere of Hicks tobacco plants grown in raw (●—●) and autoclaved (○—○) field soil for 28 days.

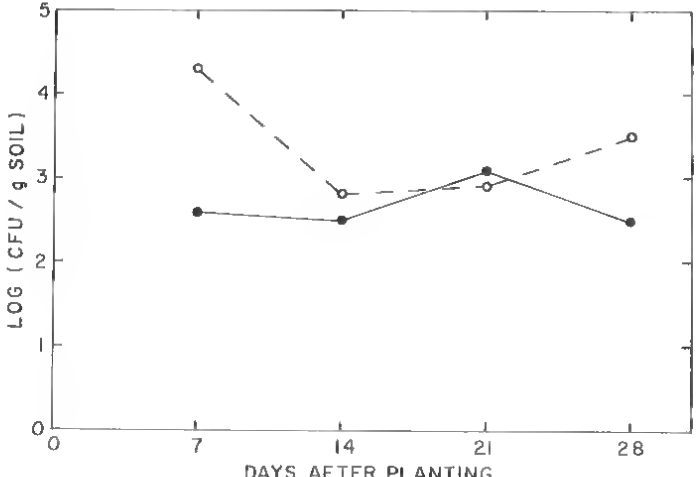

5
4
3
2
1
0
LOG (CFU / g SOIL)
0
7
14
21
28
DAYS AFTER PLANTING

Fig. 3.3. Populations of fluorescent Pseudomonas spp. associated with root surfaces of Hicks tobacco plants grown in raw (●——●) or autoclaved (○——○) field soil for 28 days.

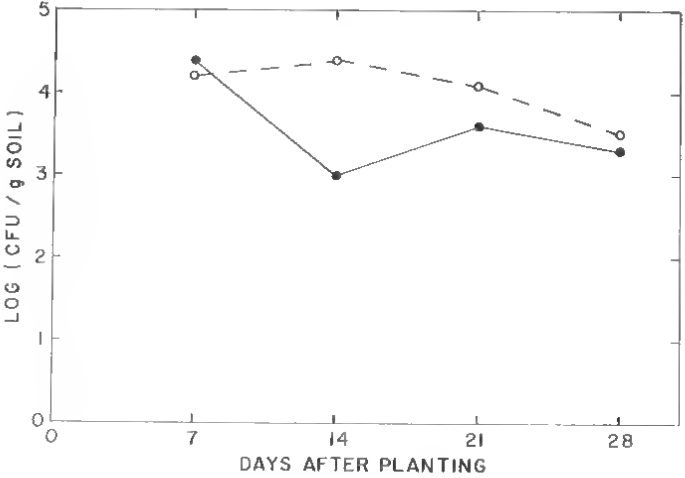

LOG (CFU / g SOIL)
0
1
2
3
4
5
0
7
14
21
28
DAYS AFTER PLANTING

Fig. 3-9. Populations of fluorescent Pseudomonas spp. in raw (●—●) or autoclaved (○—○) field soil during 28 days of growth of Hicks tobacco plants.

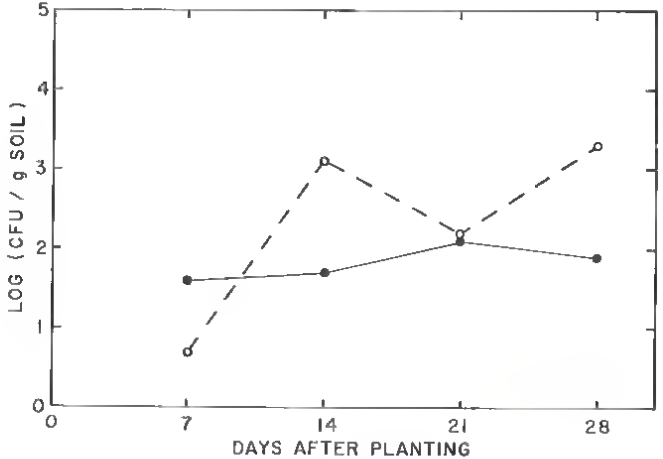

LOG (CFU / g SOIL)
0
1
2
3
4
5
0
7
14
21
28
DAYS AFTER PLANTING

Fig. 3-10. The relationships between the numbers of fungal taxa associated with the a) rhizosphere, b) root surface, and c) non-rhizosphere soil regions of Hicks tobacco plants and period of growth in raw (●—●) or autoclaved (○—○) field soil.

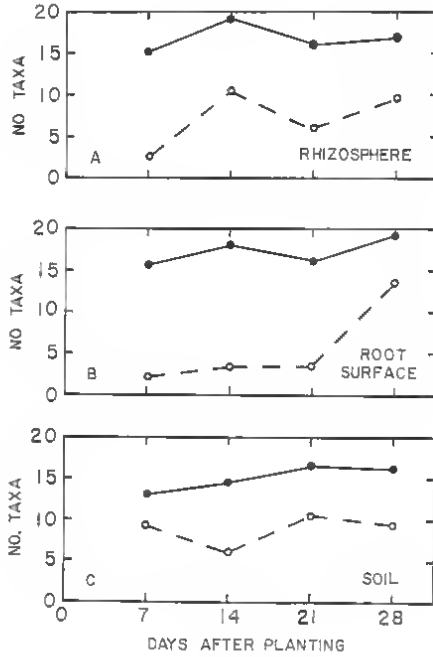

20
15
10
5
0
NO TAXA
A
RHIZOSPHERE
20
15
10
5
0
NO TAXA
B
ROOT
SURFACE
20
15
10
5
0
NO. TAXA
C
SOIL
0
7
14
21
28
DAYS AFTER PLANTING

TABLE 3-1. Populations of fungi that [illegible] rhizosphere of Hicks tobacco plants [illegible] soil for 28 days

Fungi	Propagules x 10^2/g at days after planting[a]			
	7	14	21	28
Penicillium sp.	13.9	33.5	5.9	[illegible]
Trichoderma sp.	14.1	32.5	9.4	[illegible]
Aspergillus sp.	12.8	35.7	13.0	[illegible]
Fusarium sp. and Cylindrocarpon sp.	8.1	10.8	5.2	[illegible]
Mortierella sp.	2.7	1.4	0.8	0.3
Paecilomyces sp.	2.4	3.4	1.6	1.1
Pythium sp.	2.2	0.4	0.4	0.3
Gliocladium sp.	1.4	6.4	2.2	1.5
Myrothecium sp.	1.1	1.1	0.2	0.4
Fumicladium sp.	1.1	0.7	--[b]	0.6
Talaromyces sp.	0.7	3.6	2.0	0.7
Mucoraceae	0.9	--	--	--
Cladosporium sp.	--	0.8	--	0.2
Other	5.5	12.0	0.7	1.4
Total	72.9	142.3	41.4	50.4

[a]Propagules x 10^2 per g oven-dried, rhizosphere soil estimated from numbers of colonies derived from soil suspensions plated on solidified potato dextrose agar amended with 50 mg chlortetracycline hydrochloride and 1 ml of tergitol NP-10 per liter of medium.
[b]-- = population not detectable.

TABLE 3-2. Populations of fungi that [illegible] surfaces of Hicks tobacco plants grown [illegible] days

Fungi	Propagules x 10^2/g soil at days after planting[a]			
	7	14	21	28
Penicillium sp.	20.5	25.4	42.7	7.8
Trichoderma sp.	18.4	28.9	10.7	9.1
Aspergillus sp.	21.0	32.8	18.3	4.0
Fusarium sp. and Cylindrocarpon sp.	3.1	6.0	38.7	2.2
Mortierella sp.	1.4	2.5	1.4	0.1
Fascilomyces sp.	7.6	7.5	3.3	1.0
Pythium sp	8.6	1.7	1.0	0.1
Gliocladium sp.	0.7	2.7	0.8	0.1
Myrothecium sp.	3.6	4.0	6.3	0.2
Fusicladium sp.	4.3	2.0	0.3	1.2
Talaromyces sp.	1.4	0.8	1.3	0.4
Cladasporium sp.	0.7	1.3	0.1	0.1
Pestalotia sp.	1.9	-[b]	--	
yeast	--	0.8	--	--
Alternaria sp.	--	--	--	0.1
Other	9.7	11.2	7.4	0.7
Total	103.3	127.6	132.3	28.0

[a]Propagules x 10^2 per g oven-dried, rhizosphere soil estimated from numbers of colonies derived from soil suspensions plated on solidified potato dextrose agar amended with 50 mg chlortetracycline hydrochloride and 1 ml of tergitol NP-10 per liter of medium.

[b]-- = population not detectable.

TABLE 3-3. Populations of fungi recovered from [illegible] soil during 28 days of growth of Hicks tobacco plants

Fungi	Propagules x 10^2/g soil at days after planting[a]			
	7	14	21	28
Penicillium sp.	15.7	17.1	35.8	23.9
Trichoderma sp.	22.5	23.3	40.5	25.1
Aspergillus sp.	15.1	33.7	54.0	27.3
Fusarium sp. and Cylindrocarpon sp.	3.2	16.8	18.3	13.8
Mortierella sp.	0.7	0.8	0.4	0.1
Paecilomyces sp.	0.7	3.1	6.4	1.2
Pythium sp.	0.3	0.5	0.3	0.1
Gliocladium sp.	3.2	7.0	4.8	0.8
Myrothecium sp.	0.7	0.7	2.7	1.5
Fusicladium sp.	--[b]	1.0	2.2	3.5
Talaromyces sp.	--	1.6	5.2	3.2
Mucoraceae	--	0.3	--	-
Curvularia sp.	0.4	--	--	--
yeast	--	--	--	--
Other	5.3	7.6	15.6	6.0
Total	67.8	113.5	186.2	106.4

[a] Propagules x 10^2 per g oven-dried, rhizosphere soil estimated from numbers of colonies derived from soil suspensions plated on solidified potato dextrose agar amended with 50 mg of chlortetracycline hydrochloride and 1 ml of tergitol NP-10 per liter of medium.

[b] -- = population not detectable.

Table 4. Population of fungi that colonized the rhizosphere of Hicks tobacco plants grown in autoclaved Yield soil for 28 days

Fungi	Propagules x 10^2/g soil at days after planting[a]			
	7	14	21	28
Penicillium sp.	7.2	0.7	0.4	3.1
Trichoderma sp.	1.2	<0.1	9.0	0.8
Aspergillus sp.	--[b]	<0.1	--	0.2
Fusarium sp. and Cylindrocarpon sp.	79.7	0.2	<0.1	4.4
Paecilomyces sp.	--	<0.1	--	--
Gliocladium sp.	--	<0.1	--	--
Myrothecium sp.	--	--	<0.1	--
Fusicladium sp.	--	<0.1	<0.1	0.5
Talaromyces sp.	--	--	<0.1	--
Mucoraceae	--	--	<0.1	--
Cladosporium sp.	--	<0.1	<0.1	0.7
yeast	227.3	0.8	--	7.1
Alternaria sp.	--	<0.1	--	<0.1
Other	--	<0.1	0.1	<0.1
Total	315.4	2.1	11.6	17.0

[a] Propagules x 10^2 per g oven-dried, rhizosphere soil estimated from numbers of colonies derived from soil suspensions plated on solidified potato dextrose agar amended with 50 mg of chlortetracycline hydrochloride and 1 ml of tergitol NP-10 per liter of medium.

[b] -- = population not detectable.

TABLE 3-5. Populations of fungi that colonized the root surfaces of Hicks tobacco plants grown in autoclaved [illegible] soil for 28 days

Fungi	Propagules x 10^2/g soil at days after planting[a]			
	7	14	21	28
Penicillium sp.	1.5	11.8	2.8	1.8
Trichoderma sp.	1.8	2.6	35.2	0.3
Aspergillus sp.	--[b]	--	--	0.1
Fusarium sp. and Cylindrocarpon sp.	1,075.9	--	--	1.0
Paecilomyces sp.	--	--	--	<0.1
Gliocladium sp.	--	--	--	0.1
Myrothecium sp.	--	--	--	<0.1
Fusicladium sp.	--	--	--	0.1
Cladosporium sp.	2.9	7.9	0.1	0.3
yeast	1,838.3	94.7	0.9	1.7
Alternaria sp.	--	--	--	<0.1
Other	--	2.6	0.3	<0.1
Total	2,920.4	119.6	39.3	5.6

[a] Propagules x 10^2 per g oven-dried, rhizosphere soil estimated from numbers of colonies derived from soil suspensions plated on solidified potato dextrose agar amended with 50 mg of chlortetracycline hydrochloride and [b] 1 ml of tergitol NP-10 per liter of medium.
-- = population not detectable.

TABLE 3-6. Populations of fungi that colonized autoclaved field soil during 28 days of growth of Hicks tobacco plants

Fungi	Propagules x 10^2/g soil at days after planting[a]			
	7	14	21	28
Penicillium sp.	1.1	0.8	1.1	2
Trichoderma sp.	<0.1	<0.1	<0.1	0.1
Aspergillus sp.	0.1	<0.1	0.1	<0.1
Fusarium sp. and Cylindrocarpon sp.	0.1	<0.1	0.1	<0.1
Pythium sp.	--[b]	--	<0.1	--
Gliocladium sp	<0.1	<0.1	0.1	<0.1
Myrothecium sp.	--	--	<0.1	--
Fusicladium sp.	--	<0.1	0.1	0.1
Mucoraceae	<0.1	--	<0.1	--
Cladosporium sp.	0.3	0.1	0.5	0.4
yeast	<0.1	0.1	0.7	0.2
Epicoccum sp.	--	--	--	0.2
Other	<0.1	<0.1	<0.1	<0.1
Total	1.8	2.3	2.9	3.9

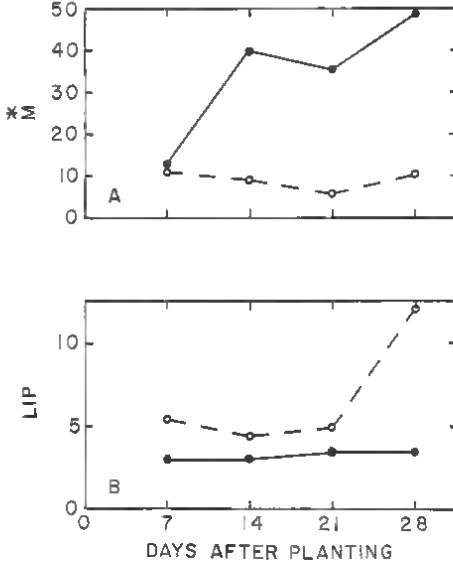
[a] Propagules x 10^2 per g oven-dried, rhizosphere soil estimated from numbers of colonies derived from soil suspensions plated on solidified potato dextrose agar amended with 50 mg of chlortetracycline hydrochloride and 1 ml of tergitol NP-10 per liter of medium.

[b] -- = population not detectable.

Fig. 3-11. The average length of fungal hyphae associated with the surfaces of first-order roots of Hicks tobacco plants grown in raw (●—●) or autoclaved (○– –○) field soil for 28 days.

CM HYPHAE /
10 CM FIRST ORDER ROOTS
0
5
10
15
20
0
7
14
21
28
35
DAYS AFTER PLANTING

Fig. 11-12. The relationship between a) mean crowding ($\overset{*}{M}$) and b) Lloyd's index of patchiness (LIP) associated with hyphal growth of fungi on surfaces of first-order roots of Hicks tobacco plants and period of plant growth in raw (●—●) or autoclaved (○—○) field soil.


50
40
30
20
10
0
*M
A
10
5
0
LIP
B
0
7
14
21
28
DAYS AFTER PLANTING

If regions colonized by hyphae along the surfaces of roots were dispersed in a random fashion, Lloyd's index of patchiness would be equal to 1. As the aggregation or clumping of colonized regions increased, values of the index would increase as well.

The aggregation of colonized regions along the surfaces of first-order roots was always greater in association with roots of plants grown in autoclaved soil as compared to raw soil (Fig. 3-12) Index values associated with fungal colonization of roots in raw soil were very consistent throughout the period of plant growth; values of this index varied between 3 and 4. Index values associated with colonization of root surface regions in autoclaved soil were less consistent and varied between 5 and 14.

Discussion

Population densities of total fungi within the rhizosphere and at the root surface of tobacco were fairly constant during 28 days of plant growth in raw field soil. Observations of constant densities of fungi associated with young plant root systems have been made previously. Van Vuurde (92) reported that the number of total fungal propagules per gram of fresh roots of spring wheat did not change during 14 days of plant growth. Hornby and Ullstrup

[illegible] observed [illegible] rhizosphere of maize remained unchanged during 77 days of plant growth in the field. Increases in population densities after this date were associated with a shift from vegetative to reproductive plant growth.

The population densities of total fungi detected in the rhizosphere of tobacco plants grown in raw soil in the present study were lower than densities which have been reported previously in association with tobacco root systems (48, 88). Timonin (88) reported densities of total fungi which varied between 240 and 940 x 10^3 propagules per gram of rhizosphere soil of two tobacco cultivars. The plants had been grown in the field and were sampled late in the growing season. In greenhouse trials population densities of total fungi in the rhizospheres of several cultivars varied between 58 and 112 x 10^3 propagules per gram of soil during 23 days of growth in field soil. The differences in densities of fungi associated with roots of tobacco in the present study and those reported by Timonin may be related to the greater physiological age of plants sampled in the earlier investigation. Additionally, densities of fungi in free soil in the earlier study were ten times greater than the population densities in raw, non-rhizosphere soil reported here. The impact of larger populations of fungi in non-rhizosphere soil on colonization of the rhizosphere of tobacco may be great

The decline in popula[illegible] of [illegible] associated with tobacco root systems during 28 days [illegible] plant growth in autoclaved soil likely occurred as [illegible] penetrated into deeper, sparsely colonized soil layers. Recolonization of autoclaved, non-rhizosphere soil by [illegible] had proceeded very slowly during this growth period.

Population densities of total fungi in autoclaved, non-rhizosphere soil were less than those reported previously during equivalent time periods after various chemical treatments (41, 54, 93). Welvaert (93) and Katznelson and Richardson (41) observed that soils treated with steam were recolonized by fungi more slowly than were soils treated with various chemicals. The slower rate of recolonization of autoclaved soil in the present study may have been related to unfavorable conditions created by treatment (80).

The diversity of a community of organisms is defined by the number of taxa present and the relative numbers of individuals within each taxon (66). Differences among the diversities of fungal communities within the various regions of ecosystems with raw and autoclaved soil were noticeable in terms of both variables. Differences in the numbers of taxa encountered within the two ecosystems were most noticeable in the rhizospheres and at the root surfaces of tobacco during the first 21 days of growth in raw or autoclaved soil. Greater numbers of taxa were observed within these regions in association with raw soil

as compared with autoclaved soil. By day [illegible] numbers of taxa detected had decreased. Relative numbers of individuals within these taxa varied between ecosystems and over time within the autoclaved soil ecosystem itself. Species of Fusarium and Cylindrocarpon as well as various yeasts initially were able to colonize rapidly the unoccupied substrates within autoclaved soil and dominate the community. With time, perhaps in response to changes in environmental conditions, their dominance within the community declined and other organisms were able to become established. The communities of fungi associated with various regions of the ecosystem with raw soil were dominated by different organisms, species of Fusarium and Cylindrocarpon and yeasts were represented in much lower relative numbers than in autoclaved soil. Within the raw soil ecosystem, species of Penicillium, Trichoderma, and Aspergillus dominated the fungal community.

Interpretation of relative numbers of propagules of various fungi was difficult in terms of contribution to total community activity. This was related to the difficulties associated with determinations of the proportion of propagules functioning actively in soil. It could not be determined readily whether the majority of propagules which gave rise to colonies on agar plates were resting structures or hyphal fragments.

The rapid establishment of numerous fungal [illegible] the rhizosphere and along root surfaces of tobacco [illegible] systems grown in raw soil agrees with observations of Zak and Parkinson (95). These authors observed a maximum rate of establishment of fungal taxa on root surfaces of slender wheat grass at the earliest sampling date of 2 weeks. In the present study, the more rapid development of diverse fungal communities associated with root systems of tobacco grown in raw soil as compared to autoclaved soil, was likely related to the greater diversity of fungi within the surrounding raw, non-rhizosphere soil. Differences in both numbers of taxa and population densities of fungi between raw and autoclaved, nonrhizosphere soils were readily apparent.

The genera of fungi associated with the rhizosphere and root surfaces of Hicks tobaco seedlings grown in raw soil were similar to those reported in association with several other plant species (23, 31, 52, 70, 82, 85). Comparisons of the diversity of the fungal communities associated with root surfaces of tobacco with diversities of such communities associated with other plant species are difficult to make because of differences in sampling methodologies. Prior to this study, evaluations of fungal communities associated with root surfaces were made by the method of Harley and Waid (31). These investigators evaluated the composition of fungal communities on the basis of frequencies of occurrance of species or genera on

1-cm segments of roots which had been plated on [illegible] nutrient medium. Roots had been rinsed serially for [illegible] prior to plating to ensure removal of viable nonhyphal propagules. In several studies in which this method was utilized (70, 82, 85), species of Cylindrocarpon, Fusarium, and Gliocladium were among the most common encountered as colonizers of root surfaces. Such organisms were encountered commonly in association with root surfaces of tobacco in the present study.

The use of frequency data in earlier investigations of root surface community structure allowed for limited estimation of coverage of these surfaces by these organisms. Several investigators reported patchy occurrences of fungal hyphae on roots of several plant species during early stages of plant growth (23, 31, 70, 82). Time of sampling in these reports corresponded generally with the sampling periods in the present study. Generally, over time the percentages of uncolonized root segments were reported to decrease until, in some cases, 100% of the root segments plated supported fungal colonization (23, 82). Whether smaller regions on the surfaces of these root segments remained uncolonized, however, could not be determined utilizing the plating technique of Harley and Waid (31).

Taylor and Parkinson (85) reported that colonizaton of broad-bean roots was continuous at all sampling dates, with the exception of a continuous, uncolonized region near the

root tip. These authors based their conclusions on [illegible] observation of 3-mm root segments, and considered a particular segment colonized if only one hyphal fragment was observed somewhere along its length. The use of direct microscopic observation in the present study allowed more detailed evaluation of fungal colonization of root surfaces of tobacco. Examination of fungal colonization along surface segments bounded by a Whipple disc 700 μ in diameter revealed that colonization of root surfaces by fungi was obviously patchy during 28 days of plant growth in both raw and autoclaved soils.

It was not possible to distinguish between species or genera of fungi developing along root surfaces by the use of microscopic observation. The spatial relationships between colonizing fungal species within this region are therefore unknown. However, evidence provided in earlier root plating experiments suggested that early in the process of root system development, occupation of a surface region by one fungal species precluded colonization of that same region by other fungal species (23, 31, 82). As root development continued, the percentage of regions colonized by more than one fungal species increased.

Lloyd's indices of mean crowding and patchiness were derived originally to describe relative aggregations of individuals within animal populations (47). He suggested that these descriptive parameters were valid only if the individuals within a population were relatively rare in

reduction to the extent of available habitat and that the habitat itself was relatively continuous and uniform. Although it is difficult to define an individual within a population of a fungus, it is possible to envision the thallus of a septate fungal species as being composed of densely aggregated, individual cells. Initial estimates of the aggregation of fungi, as a composite of one or more taxa, along the relatively continuous habitat of root surfaces might be developed utilizing this approximation to individuals.

The much larger proportion of microscope fields occupied by fungal hyphae and the greater average length of hyphae along root surfaces within raw soil as compared to autoclaved soil suggested more extensive coverage of root surfaces in the former soil environment. Lloyd's index of patchiness provided a parameter which described the manner in which these occupied fields were dispersed. Values of Lloyd's index of patchiness for both raw and autoclaved soils were greater than one and thus implied greater aggregation than expected if fungal colonization were random. Consistently, larger values of this index associated with first-order roots growing in autoclaved, sparsely colonized soil suggested a patchier occurrence of colonized regions along the surface of roots in this environment as compared to first-order roots within more densely colonized raw soil. Observations of denser configurations of fungal hyphae within colonized regions of

root surfaces [illegible] were supported by consistently greater values of Lloyd's index of mean crowding associated with the former soil environment.

Pielou (71) states that mean crowding and Lloyd's index of patchiness are independent of the mean, m. However, examination of the definitions of these indices reveals that both descriptive parameters are dependent on the mean number of hyphal intersects per microscope field, and therefore average hyphal length. It is not surprising that the relative values of mean crowding and Lloyd's index of patchiness in the raw and autoclaved soil ecosystems varied directly with relative values of average hyphal lengths in the two soil ecosystems.

Thus, the development of a complete picture of fungal colonization of surfaces of first-order roots requires consideration of the proportion of occupied microscope fields and average hyphal lengths as well as mean crowding and Lloyd's index of patchiness. The use of such quantitative measures provides at least initial descriptions of site occupation. The development of such base line information is necessary if it is desired to evaluate the influence of imposed treatments on ecosystem behavior

CHAPTER IV
THE INFLUENCE OF AN INTRODUCED COMPOSITE OF MICROBIAL ANTAGONISTS ON INFECTION OF TOBACCO BY PHYTOPHTHORA PARASITICA VAR. NICOTIANAE AND DEVELOPMENT OF BLACK SHANK

Introduction

Many attempts have been made to control diseases caused by soilborne pathogens through manipulations of single microbial antagonists (13). Such attempts have met with variable success. Very often initial selections of antagonists have been based on such expressions of antagonism as inhibition of pathogen growth or hyperparasitism in vitro. Less emphasis has been placed on the abilities of selected antagonists to express desirable antagonistic traits in situ. An additional important criterion in the selection of antagonistic microorganisms is the ability of such organisms to colonize rapidly and stably the niches of critical importance to pathogen activity within soil or in association with host plants; selections of other antagonistic characteristics might better be made secondarily to this criterion. Rapid and stable colonization of such critical sites might be achieved efficiently by the manipulation of a composite of microorganisms which, as a group, would be resistant to fluctuating environmental conditions.

The concept that a biological pest might be eliminated from its normal niche within an ecosystem by manipulations of other community components of that ecosystem is not new (66). Recently, Marois and Mitchell (53, 54) were able to reduce the incidence of crown rot of tomato, incited by Fusarium oxysporum f. sp. radicis-lycopersici, through the addition of four isolates of antagonistic fungi to freshly fumigated soil. These combined isolates colonized the unoccupied soil rapidly in succession and reduced the build-up of saprophytic populations of this pathogen and subsequently incidence of plant infection.

Such an approach may be appropriate as a method of control of black shank of tobacco (Nicotiana tabacum L.) which is incited by Phytophthora parasitica Dast. var. nicotianae (Breda de Haan) Tucker. Initial populations of this pathogen in non-rhizosphere soil are very low and highly aggregated (20, 39). The pathogen is capable of little, if any, saprophytic growth. Thus, it would be difficult to attain sufficient interactions between populations of introduced antagonists and the pathogen to attain significant reductions of inoculum density in non-rhizosphere soil.

Populations of the pathogen, however, do build up rapidly in association with developing root systems of tobacco (22, 39). Infection of tobacco roots occurs predominantly in the restricted zone of elongation just behind the apical meristem (50, Chapter II). Increases in

population densities in the rhizosphere likely are the result of secondary inoculum production, including spores and zoospores, on the surfaces of infected roots. It is within the rhizosphere of tobacco, in particular in the regions of root tissues susceptible to infection, where interactions between populations of P. parasitica var. nicotianae and microbial antagonists are likely to be most effective in limiting disease.

Investigations were established to evaluate colonization of critical sites by an introduced composite of competitive microorganisms capable of rapid colonization of regions around tobacco root systems. Of particular interest initially was the contribution of such site occupation to reductions of infections of tobacco roots by the pathogen. The influence of this composite of antagonists on long term development of black shank also was examined.

Materials and Methods

Trials were established in a plant growth room to evaluate the ability of an introduced composite of fungi and bacteria to colonize sites susceptible to infection by P. parasitica var. nicotianae within root systems of tobacco and to reduce the number of early infections of a susceptible tobacco cultivar. Competition for occupation of such sites was evaluated in steole and disrupted soil

[illegible] respectively. The composite was comprised of fungi and bacteria which were known to colonize developing root systems of tobacco in raw field soil rapidly and stably over time. Single, randomly selected isolates of *Trichoderma harzianum* Rifai, *Aspergillus carbonarius* (Bainier) Thom, *A. terreus* Thom, *Penicillium steckii* Zaleski, and *Pseudomonas putida* (Trevisan) Migula were selected without evaluation of other antagonistic characteristics.

Each fungal isolate was grown on potato dextrose agar for 2 weeks at 25 C and 12 hours of light (300 $\mu Ein/m^2/sec$) per day. Conidia were washed from the surfaces of colonies and concentrations were determined from counts in 20 haemocytometer fields. The isolate of *P. putida* was grown on King's medium B (42) for 24 hours at 25 C in the dark, and the bacterial cells then were pelleted by centrifugation, washed, and resuspended in 0.1 mM $MgSO_4$. Densities of bacteria in suspensions were determined from the percentages of transmission of light at 600 nm through suspensions and subsequent interpolations along a curve of standardized light transmission versus bacterial cell density.

Conidia and bacterial cells of each isolate were combined in suspension before amendment of soil; raw or autoclaved field soil (Blichton sand) was amended with a composite of 1×10^5 conidia and bacterial cells of each isolate per gram of soil. Soil had been prepared for use as described in Chapter II.

Suspensions of chlamydospores of isolate B-730 of *P. parasitica* var. *nicotianae* were prepared as described in Chapter II. Chlamydospores were added to composita-amended, raw and autoclaved soils to establish an inoculum density of 50 chlamydospores per gram of soil. Chlamydospores also were added to non-amended, raw or autoclaved soil at the same inoculum density. Amended soil infested with the pathogen was added to 100-ml, polypropylene beakers according to the infested soil layer method described in Chapter II. However, the upper soil layer which was not infested with propagules of the pathogen had been amended with propagules of the introduced composita to match the conditions of the center, infested soil layer. A 2-week-old Hicks tobacco seedling was transplanted into the noninfested soil layer of each container. Fifteen seedlings were transplanted in this manner in both amended and non-amended, raw and autoclaved soils infested with *P. parasitica* var. *nicotianae*. Control treatments consisted of six seedlings planted singly into polypropylene beakers containing amended or non-amended, raw or autoclaved soil not infested with the pathogen. Transplanted seedlings were maintained in watering trays and covered with clear plastic in a plant growth room at 25±2 C under 16 hours of light (700 uEin/m^2/sec) per day. Plants were watered from below by flooding trays to a depth of 1 cm for about 3 min on alternate days.

Ten seedlings at intervals of tobacco roots were evaluated after 14 days of plant growth in each soil ecosystem. Fifteen asymptomatic seedlings were removed from both amended and non-amended, raw and autoclaved soils which had been infested with F. parasitica var. nicotianae. Ten of seedlings were removed, and root systems were surface-disinfested by dipping in 70% ethanol and rinsing in deionized water. Each root system was dissected according to the classification scheme of the morphometric root analysis system (21) and root segments were plated on selective medium as described in Chapter II.

Colonization of the rhizospheres and root surfaces of tobacco plants, as well as of the non-rhizosphere soil, by the composite of fungi and bacteria was evaluated. Ten seedlings were removed from amended and non-amended, raw or autoclaved soil; root systems were bulked by treatment. Populations of introduced species as well as other fungi and bacteria were estimated in the various regions of each soil ecosystem as described in Chapter III.

Six additional seedling root systems from each amended soil infested with F. parasitica var. nicotianae, and three seedling root systems from each soil not infested with the pathogen were evaluated for root system development as described in Chapter II. Colonization of surfaces of first-order roots of three of these same seedlings by fungi within each soil ecosystem was evaluated as described in Chapter III. Influences of treatments on infection and host

[illegible] were evaluated by analysis of variance within each trial. Since contrasts were selected after examining experimental outcomes, appropriate contrasts between treatments within individual trials were made using Scheffe's intervals (25). Comparisons of corresponding treatment effects between trials were made using Student's two-sample t test (25). Trials of competition between composite organisms and _F. parasitica_ var. _nicotianae_ in the plant growth room were conducted twice.

Further evaluations were made of the influences of the introduced composite of microorganisms on populations of the pathogen in non-rhizosphere soil. Raw or autoclaved field soil was infested with 50 chlamydospores of the pathogen per gram of soil. Each infested soil was amended with a composite of 1×10^5 conidia and bacterial cells of each composite isolate per gram of soil or was left non-amended. One-kilogram samples of soil amended with composite isolates and infested with the pathogen were moistened to 15% gravimetric soil moisture content and placed into individual closed plastic containers with small holes to allow air exchange. Containers were weighed daily and deionized water was added as needed to maintain constant soil moisture. Periodically during a 56-day period, a single soil sample was taken from each treatment combination to a depth of 3 cm using a surface-disinfested cork borer. Samples were diluted appropriately and plated onto media selective for general fungi, fluorescent _Pseudomonas_ spp., and

Phytophthora spp. as described in Chapter II. Estimates of population densities of fungi and bacteria were derived from the average numbers of colonies of each organism on 10 plates of each medium. At each sampling date, mean densities of P. parasitica var. nicotianae in the four trials were compared by Tukey's multiple comparison procedure for honestly significant differences (25). Evaluations of interactions between populations of microbes in the composite and P. parasitica var. nicotianae in non-rhizosphere soil were performed four times.

The influence of the introduced composite of antagonists on long term development of black shank was evaluated in glasshouse trials. Two hundred grams of amended or non-amended, raw or autoclaved field soil were infested with 5 chlamydospores of the pathogen per gram of soil and were layered over autoclaved boilder's sand in individual 10-cm pots. Over this was layered 200 g of raw or autoclaved soil amended with 1×10^5 propagules of each composite isolate. A single, 4-week-old, Hicks tobacco plant was transplanted into each pot. Control treatments consisted of Hicks seedlings transplanted into raw or autoclaved soil which had or had not been amended with propagules of the composite but was not infested with P. parasitica var. nicotianae.

Plants were maintained in a glasshouse for 90 days at 16 to 30 C and were watered from above on alternate days; half-strength Hoagland's solution (33) was added in place of

water twice [illegible] week. Plants were examined every [illegible] for expression of symptoms of black shank, including wilting and stem discoloration. To confirm infection by P. parasitica var. nicotianae, symptomatic plant root systems were removed from soil, surface-disinfested by dipping in 70% ethanol, rinsed in deionized water and plated onto selective medium (38). Trials were conducted three times. Influences of amendments of composites in soil on mortality of Hicks tobacco after 90 days of growth were evaluated by analysis of variance. Proportions of diseased were transformed by arcsine square-root and appropriate contrasts between treatment means were made using Scheffe's intervals (25).

Results

Amendment of raw or autoclaved field soil with a composite of microbial isolates was associated with large increases in propagule densities of fungi and fluorescent Pseudomonas spp. within the rhizosphere and root surfaces of Hicks tobacco plants after 2 weeks of growth. Large increases in densities of these organisms were noted in non-rhizosphere soils as well. Absolute densities and relative increases in densities of fungi were quite variable in association with soil amendment. Population densities of total fungi within the various regions of the ecosystem

with autoclaved soil were between 500 and 4000 times greater than densities of total fungi in non-amended, autoclaved soil in two trials (Table 4-1). Densities of total fungi in regions of the ecosystem with raw soil increased only two- to seven-fold in association with soil amendment. Within various regions of the ecosystem with non-amended, autoclaved soil, recoverable propagules of fungal species belonging to microbes in the composite represented between 0 and 65% of the total population of fungi (Table 4-2). Within amended, autoclaved soil the percentages of recoverable propagules of total fungi in various regions comprising introduced species was greater than 85%. In non-amended, raw soil propagules of fungal species within the composite accounted for 55 to 65% of the population density of total fungi in all regions. Amendment of raw soil with propagules of these isolates increased the contribution of the species to the total fungal community; more than 75% of recoverable propagules were of species introduced in the composite of antagonists. Other fungi observed commonly within various regions of this ecosystem included Fusarium spp., Cylindrocarpon spp., and other species of Penicillium and Trichoderma.

Population densities of fluorescent Pseudomonas spp. in various regions of raw and autoclaved soils not amended with the composite were quite variable (Table 4-3). Densities of fluorescent Pseudomonas spp. in various regions of amended soils increased between 3 and 75 times within autoclaved

soil and between 3 and 300 times in raw soil as [illegible] densities of these bacteria in respective regions of non-amended soils.

Population densities of introduced fungi and fluorescent Pseudomonas spp. associated with roots of Hicks tobacco after 2 weeks of growth in ecosystems with amended, autoclaved and raw soil generally were not maintained at the levels initially established (Tables 4-3, 4-4, 4-5) After 2 weeks of plant growth in amended, autoclaved soil, T harzianum dominated the community of fungi within the rhizosphere or in non-rhizosphere soil. The community of fungi associated with root surfaces was not dominated by any particular fungal species, most introduced species were represented fairly equally. However, A. carbonarius was not detected in association with root surfaces in this soil ecosystem in either trial. Within the rhizospheres and at root surfaces of Hicks tobacco plants grown in amended, raw soil, T. harzianum was the least commonly recovered fungal species (Table 4-5). Communities in all regions of raw soil were comprised of fairly equivalent population densities of A. terreus, A. carbonarius, and P. steckii.

Amendment of soil with the composite was not associated with any alterations in patterns of colonization of surfaces of first-order roots by fungal hyphae (Table 4-6). Average lengths of hyphae and mean crowding were greater in association with roots from raw soil than from autoclaved soil. Conversely, Lloyd's index of patchiness was greater

in assoc[illegible] roots from autoclaved soil [illegible] soil. Amendment of soils did not alter significantly values of these parameters within soil ecosystems. Absolute values of parameters did vary, however, between the two trials.

The patterns of infection by P. peresitica var. nicotianae of Hicks tobacco seedlings grown in infested raw or autoclaved soil were not altered significantly by amendment of these soils with the composite of fungi and bacteria (Table 4-7). Percentages of plants infected in two trials varied between 60 and 100% and were not correlated with any particular treatment combinations. In all treatment combinations, the majority of infections occurred on first-order roots. The average numbers of infected roots observed per infected seedling grown in amended, raw soil were less than the numbers observed per infected seedling within non-amended, raw soil in both trials; however, differences were not significant ($p=0.05$). The numbers of infected roots observed per infected seedling in autoclaved soil were not altered significantly by soil amendement in either trial ($p=0.05$). In a similar manner, inoculum efficiencies did not vary significantly in association with any treatment combination.

Analyses of patterns of root growth were restricted to elements within the first-order and second-order classes (Tables 4-8 and 4-9). Roots within higher orders previously had been noted to form very late during 14 days of plant growth (Tables 2-1 and 2-2). The numbers and total lengths

of third-order and fourth-order roots within this trial also were quite variable in association with late development of elements within the fourth-order class in particular. In many treatment combinations fourth-order roots had not appeared by the end of the period of plant growth (Tables 4-8 and 4-9).

Comparisons were made between estimated values of root growth parameters of infected and healthy seedlings. As determined by analysis of variance, amendment of soil with the composite of microbial isolates was not associated with any changes in root system development. The numbers (Table 4-8) and total lengths (Table 4-9) of first-order and second-order roots per seedling varied significantly within both trials in association with both seedling infection and soil treatment. Patterns of change in root system development in association with these two main effects were variable by trial. Changes in development were observed to be consistent within both trials only in association with seedling infection, the influence of soil treatment on values of parameters was variable by trial. The average numbers and total lengths of first-order and second-order roots of healthy plants were significantly greater than the numbers and lengths of these roots of infected plants when summed over ecosystems with raw and autoclaved soil ($p=0.05$). A significant interaction between seedling infection and soil treatment was observed in relation to the numbers and total lengths of first-order and second-order

roots per seedling only in trial 1. Within this classification significant reductions were noted in [illegible] of these growth parameters of infected plants as compared to healthy plants only as averaged over amended and non-amended, raw soils ($p=0.05$). Within this same trial no significant differences in these parameters of growth were observed between infected or healthy seedlings grown in amended or non-amended, autoclaved soil. Within trial 2, no significant differences in estimates of these parameters were observed, although values were lower in association with infected seedlings than non-infected seedlings in all ecosystems with amended or non-amended soil. The average total lengths of all roots per seedling varied significantly in association with various treatments in the same manner as the total lengths of first-order and second-order roots per seedling (Table 4-9). In contrast to the patterns of variations of numbers and total lengths of first-order and second-order roots per seedling, the average lengths of these roots per seedling did not vary significantly in association with any treatment combination (Table 4-10).

Significant differences in estimates of growth parameters were observed between corresponding treatments of trial 1 and trial 2. Such differences were not correlated with any particular treatment combinations. Within trial 1 observed average numbers and total lengths of first-order and second-order roots of plants within the majority of treatment combinations fell outside the 95% confidence

intervals of expected values for [illegible] (Table 2-8). Few parameter estimates within [illegible] fell outside of these intervals.

Amendment of raw and autoclaved, non-rhizosphere soils with a composite of microbial isolates did not influence the patterns of change in population densities of P. parasitica var. nicotianae over a 56-day period (Fig. 4-1). As determined from the average of four trials, densities of the pathogen in both amended and non-amended, non-rhizosphere soils declined steadily over time after brief initial increases. Maximum densities within each treatment were attained between day 2 and day 7 and did not differ significantly as determined by Tukey's multiple comparison procedure for honestly significant differences ($p=0.05$). After the period of increase in population densities, the numbers of propagules of P. parasitica var. nicotianae declined in all soil ecosystems. Densities of the pathogen in the four ecosystems did not differ significantly at any sampling date. Viable propagules of the pathogen remained in each soil after 56 days. At the end of each trial, five, two-week-old Hicks tobacco seedlings were transplanted into infested soils. In each trial all seedlings in all soil treatments died from black shank.

During the 56-day period, populations of introduced fungi either remained constant or increased in amended, raw and autoclaved soils (Fig. 4-2 and 4-3). Within amended, raw soil no single isolate obviously dominated the community of

soilborne fungi (Fig. 4-2). Population densities of each introduced fungal species remained relatively stable between 10,000 and 52,000 propegules per gram of soil, the numbers of recoverable propagules of each species from day 0 onward represented only about 10 to 50% of the numbers initially added to raw soil. Population densities of composite fungi in non-amended, raw soil were much lower and varied between only 350 and 10,000 propagules per gram of soil for individual species; these fungi accounted for only about 35 to 48% of recoverable fungal propegules.

Within amended, autoclaved soil, population densities of Trichoderma harzianum increased steadily over time and dominated the fungal community (Fig. 4-3). Densities of this fungus increased from about 400,000 propagules per gram of soil at day 0 to 5.5×10^6 propagules per gram of soil at day 56. Densities of other introduced species varied between only 12,000 and 51,000 propagules per gram of soil. Introduced fungi accounted for more than 96% of propagules of total fungi at all sampling dates. Population densities of community members in non-amended, autoclaved soil were extremely variable and accounted for anywhere from 1 to 87% of the total population of fungi detected at any single sampling date. No pattern was observed in such fluctuations over time. Densities of individual species within the composite fluctuated between 0.5 and 1,700 propagules per gram of soil. No obvious patterns of dominance by individual composite members were observed over time.

The addition of a composite of fungi and bacteria to raw and autoclaved, non-rhizosphere soil was associated with large increases in numbers of colony forming units (CFU) of fluorescent Pseudomonas spp. (Fig. 4-4). Population densities of these organisms in amended and non-amended soils increased initially through day 4 or 7 and then began to decline. Fluctuations in densities of fluorescent Pseudomonas spp. were least severe in non-amended, raw soil; densities in non-amended, autoclaved soil initially were similar, but these continued to increase rapidly (about two log units). After 56 days the numbers of colony forming units of fluorescent Pseudomonas spp. per gram of amended, raw or autoclaved soil were still greater than in respective non-amended soils. Amendment of autoclaved soil with bulked isolates was associated with increases in population densities of fluorescent Pseudomonas spp. to levels greater than those established originally. Within raw soil densities declined to less than 10% of the numbers established originally.

During 90 days of Hicks tobacco growth in infested soils in a glasshouse, black shank developed more slowly in amended or non-amended, raw soil than in either autoclaved soil ecosystem (Table 4-11). Non-amended, raw soil appeared to be suppressive, mortality of tobacco within this soil ecosystem was significantly less than mortality of tobacco grown in non-amended, autoclaved soil. Average mortalities of tobacco after 90 days of growth in three trials were less

in amended, raw or autoclaved soil than in corresponding non-amended soils; the differences in mortalities, however, were not significant. At the end of the growth period, P parasitica var. nicotianae was isolated from all root systems of remaining live plants in amended, autoclaved soil. The pathogen was not isolated from roots of any remaining asymptomatic plants from amended or non-amended raw soil. The times required to attain 10% plant mortality, and to increase from 10 to 50% mortality were similar in amended and non-amended, autoclaved soils. The time required for increase in mortality from 10 to 90% in non-amended, autoclaved soil was 53 days, there were insufficient increases in black shank within amended, autoclaved soil to estimate the period of increase from 10 to 90% mortality. Estimates of these parameters describing disease development could not be developed for tobacco grown in either ecosystem with raw soil because of insufficient mortality data.

TABLE 4-1. The influence of an introduced composite of fungi and bacteria on population densities of total fungi associated with the rhizospheres and root surfaces of Hicks tobacco and non-rhizosphere soil after 14 days of plant growth in raw or autoclaved soil

Trial	Soil	Antagonists	Propagules x 10^2 of total fungi per g soil or roots[a]		
			Rhizosphere	Root Surface	[illegible]
1	Autoclaved	-[b]	1.5	4.3	[illegible]
		+	5,666.1	2,076.8	4,320.0
	Raw	-	101.2	90.8	210.5
		+	419.7	278.6	822.5
2	Autoclaved	-	3.0	7.7	2.7
		+	1,950.0	93.8	2,553.0
	Raw	-	146.3	112.8	382.5
		+	297.0	237.5	2,847.0

[a] Propagules x 10^2 per gram of oven-dried soil or roots estimated from numbers of colonies derived from soil or root-surface suspensions plated on solidified potato dextrose agar amended with 50 mg chlortetracycline hydrochloride and 1 ml Tergitol 10-NPX per liter of medium.

[b] - = composite of antagonists was not added to soil, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and 10^8 bacterial cells of Pseudomonas putida were added per gram of raw [illegible] autoclaved soil.

TABLE 4-2. The relationship between soil amendment with a composite of fungi and bacteria and the percentages of fungal communities associated with various regions comprising fungal species belonging to that composite after 14 days of plant growth

Trial	Soil	Antagonists	Percentage of fungal community comprised of introduced species[a]		
			Rhizosphere	Root Surface	Soil
1	Autoclaved	-[b]	65.4	0.0	38.5
		+	94.1	85.2	100.0
	Raw	-	58.5	57.5	56.5
		+	89.7	94.2	86.5
2	Autoclaved	-	14.0	48.1	6.4
		+	100.0	100.0	100.0
	Raw	-	62.9	64.0	62.1
		+	85.2	92.1	78.0

[a] Percentage of population density of total fungi comprised of introduced fungal species.

[b] - = composite of antagonists was not added to soil, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii and 10^6 bacterial cells of Pseudomonas putida were added per gram of raw or autoclaved soil.

TABLE 4 3. The influence of an introduced composite of fungi and bacteria on population densities of fluorescent Pseudomonas spp. associated with the rhizospheres and root surfaces of Hicks tobacco and non-rhizosphere soils after 14 days of plant growth in raw or autoclaved soil

Trial	Soil	Antagonists	CFU x10^2 fluoreacsnt Pasudomonas spp. g soil or roots[a]		
			Rhizosphere	Root Surfece	Soil
1	Autoclaved	-[b]	60.4	36.6	38.6
		+	1,969.5	1,903.8	1,680.0
	Raw	-	3.3	44.8	4.[illegible]
		+	194.4	342.9	195.[illegible]
2	Autoclaved	-	17.6	264.0	6.[illegible]
		+	693 8	925.0	483.0
	Raw	-	24.0	10.0	3.8
		+	67.2	306.2	54.[illegible]

[a]Colony forming units x 10^2 per gram oven-dried soil or roots estimated from numbers of colonies derived from soil or root-surface suspensions plated onto modified King's medium B (77).

[b]- = Composite of antagonists was not added to soil, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and 10^5 bacterial cells of Pseudomonas putida were added per gram of raw or autoclaved soil

TABLE 4-4. The influence of an introduced composite of fungi and bacteria on populatisu densities of fungal species within that composite associated with the rhizospheres and root surfaces of Hicks cultivar of tobacco and non-rhizosphere soil after 14 days of plant growth in autoclaved soil

Trial	Antagonists	Fungi	Propagules x 10^2 /g soil[a]		
			Rhizosphere	Root Surface	[illegible]
1	-[b]	Trichoderma harzianum	0.9	—[c]	0.3
		Aspergillus carbonarius	<0.1	—	—
		A. terreus	<0.1	—	[illegible]
		Paulcillium steckii	<0.1	--	[illegible]
		Other	333.3	4.3	[illegible]
	+	T. harzianum	4,545.0	692.3	3,280.0
		A. carbonarius	454.5	—	720.0
		A. terreus	121.2	538.4	320.0
		P. steckii	212.1	538.4	—
		Other	0.5	307.7	—

TABLE 4-4. Continued

Trial	Antagonists	Fungi	Propagules x 10^2 /g soil[a]		
			Rhizosphere	Root Surface	[illegible]
2	-[b]	Trichoderma harzianum	0.1	0.1	[illegible]
		Aspergillus carbonarius	0.1	[illegible]	[illegible]
		A. terreus	0.2	[illegible]	[illegible]
		Penicillium steckii	[illegible]	0.9	[illegible]
		Other	2.6	4.9	[illegible]
	+	T. harzianum	1,593.8	100.0	[illegible]
		A. carbonarius	206.3	[illegible]	[illegible]
		A. terreus	112.5	200.0	241.5
		P. steckii	37.5	200.0	34.5
		Other	--	--	[illegible]

[a] Propagules x 10^2 per gram of oven-dried soil or roots estimated from numbers of colonies derived from soil or root-surface suspensions plated on solidified potato dextrose agar amended with 50 mg chlortetracycline hydrochloride and 1 ml of tergitol 10-NPX per liter of medium.

[b] - = composite of antagonists was not added to soil, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarious, Penicilllum steckii, and 10^5 bacterial cells of Pseudomonas putida were added per gram of autoclaved soil.

[c] -- = population not detectable.

TABLE 4-5 The influence of an introduced composite of fungi and bacteria on population densities of fungal species within that composite associated with the rhizosphere and root surfaces of Hicks cultivar of tobacco and non-rhizosphere soil after 14 days of plant growth in raw soil

Trial	Antagonists	Fungi	Propagules x 10^2 /g soil[a]		
			Rhizosphere	Root Surface	[illegible]
1	-[b]	Trichoderma harzianum	0.8	0.5	[illegible]
		Aspergillus carbonarius	23.4	10.9	21.8
		A. terreus	35.0	38.6	93.5
		Penicillium steckii	--	2.3	0.[illegible]
		Other	42.3	38.7	90.[illegible]
	+	T. harzianum	26.2	5.4	141.[illegible]
		A. carbonarius	180.5	107.1	253.8
		A. terreus	121.9	9.1	220.0
		P. steckii	47.8	58.9	89.3
		Other	43.2	16.1	117.5

TABLE 4-5. Continued

Trial	Antagonists	Fungi	Propagules x 10^2 /g soil[a]		
			Rhizosphere	Root Surface	Soil
2	[b]	Trichoderma harzianum	2.3	--[c]	[illegible]
		Aspergillus carbonarius	22.5	11.7	35.4
		A. terreus	66.7	57.8	187.8
		Penicillium steckii	0.6	2.8	19.0
		Other	54.3	40.6	148.8
	+	T. harzianum	9.8	--	260.4
		A. carbonarius	123.3	43.8	833.3
		A. terreus	85.5	106.2	781.4
		P. steckii	46.2	68.7	347.2
		Other	32.2	18.7	625.6

[a] Propagules x 10^2 per gram of oven-dried soil or roots estimated from numbers of colonies derived from soil or root-surface suspensions plated on solidified potato dextrose agar amended with 50 mg chlortetracycline hydrochloride and 1 ml of tergitol

[b] 10-NFX per liter of medium
- = composite of antagonists was not added to soil, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and 10^9 bacterial cells of Pseudomonas putida were added per gram of raw soil.

[c] -- = population not detectable.

TABLE 4 6. The influence of an introduced composite of fungi and bacteria on fungal colonization of surfaces of first-order roots of Hicks cultivar tobacco after 14 days of plant growth in raw or autoclaved field soil

Trial	Soil	Antagonists	Length of Hyphae (cm)/ 10 cm of First-Order Roots[a]	Mean Crowding[b]	LIP[c]
1	Autoclaved	-[d]	0.8	8.9	13.9
		+	0.4	4.1	15.9
	Raw	-	16.2	55.3	5.7
		+	11.2	51.2	7.6
2	Autoclaved	-	1.0	5.4	10.9
		+	1.6	11.6	10.9
	Raw	-	8.8	34.1	8.1
		+	7.2	37.9	8.4

[a] Average lengths of hyphae were estimated using the line intersect method of Tennant (86), values are the averages of numbers of hyphal intersects with reticule grid lines in up to 100 microscope fields selected along the lengths of first-order roots of three seedlings.

[b] Mean crowding = $\bar{m} = \bar{m}^* + m/k$,

[c] LIP = Lloyd's index of patchiness = $\bar{m}/m = 1 + (1/k)$.

[d] + = composite of antagonists was not added to soil; + = 10^5 conidia of each of Trichoderma herzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and 10^8 bacterial cells of Pseudomonas putida were added per gram of raw or autoclaved soil.

TABLE 4-7. The influence of an introduced composite of fungi and bacteria on observed infectious caused by Phytophthora parasitica var. nicotianae on roots of Hicks cultivar of tobacco and inoculum efficiency after 14 days of plant growth in raw or autoclaved field soil infested with 50 chlamydospores of the pathogen per gram of soil

Trial	Soil	Antagonists[d]	Infected Roots[e]					
			Root Order				Seedling	Inoculum Efficiency[c]
			1[b]	2	3	4		
1	Autoclaved	-	5.9	1.0	0.2	0.0	7.1	0.001
		+	4.3	0.8	0.2	0.0	5.3	0.001
	Raw	-	8.3	2.2	0.4	0.0	10.9	0.003
		+	5.7	0.9	0.3	0.0	6.9	0.001
2	Autoclaved	-	7.7	2.2	0.4	0.1	10.4	0.003
		+	16.7	2.5	0.5	0.1	19.8	0.006
	Raw	-	10.3	2.5	0.6	0.1	13.5	0.004
		+	3.0	0.6	0.2	0.0	3.8	0.004

[e] Numbers of infected roots per root order or individual seedling were determined as the average of up to 15 asymptomatic, infected root systems which had been dissected completely by root order and plated onto selective medium (3%).

[b] Root orders are as defined in the morphometric root analysis system (21).

[c] Efficiencies were determined from the average of the ratios of numbers of infected roots per seedling to total number of chlamydospores (approximately 3,250 chlamydospores) within the volume of soil containing each seedling

[d] - = composite of antagonists was not added to soil, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and 10^7 bacterial cells of Pseudomonas putida were added per gram of raw or autoclaved soil

TABLE 4-8. The influence of an introduced composite of fungi and bacteria on the relationship between infection of Hicks cultivar tobacco by Phytophthora parasitica nicotianae and numbers of roots per seedling after 14 days of plant growth in raw or autoclaved field soil artificially infested with propagules of the pathogen

Trial	Soil	Antagonists	Chlamydospores/ g Soil	Number of Roots Root Order 1[a]	2	3	[illegible]
1	Autoclaved	-[b]	0	36.7x[c,d]	5.0x	[illegible]	[illegible]
			50	29.5x	4.7x	[illegible]	[illegible]
		+	0	28.3x	4.3x	[illegible]	[illegible]
			50	27.5x	5.7x	[illegible]	[illegible]
	Raw	-	0	50.3x	6.7x	[illegible]	[illegible]
			50	26.0x	3.7x	[illegible]	[illegible]
		+	0	51.7x	8.7x	[illegible]	[illegible]
			50	27.3x	4.3x	[illegible]	[illegible]

TABLE 1-2. Continued

Trial	Soil	Antagonists	Chlamydospores/g Soil	Number of Roots Root Order[a]			
				1	2	3	4
2	Autocleved	-[b]	0	47.0x[c,d]	11.0y	2.0	0.5
			50	36.2x	5.8x	1.7	0.3
		+	0	65.3y	9.0y	1.0	0.0
			50	32.5x	5.7x	1.5	0.5
	Raw	-	0	40.0x	4.7x	1.0	0.0
			50	27.3x	4.2x	1.0	0.0
		+	0	43.7x	5.7y	1.3	0.3
			50	39.7x	6.3x	1.7	0.7

[a] Root orders are as defined in the morphometric root analysis system (21).

[b] - = composite of antagonists was not added to soil; + = 10^5 conidia of each of *Trichoderma harzianum*, *Aspergillus terreus*, *A. carbonarius*, *Penicillium steckii*, and 10^5 bacterial cells of *Pseudomonas putida* were added per gram of raw or autoclaved soil

[c] Noninfested soil values are the averages of numbers of elements per root order of three healthy seedlings; infested soil averages are from six infected root systems

[d] Values within corresponding root orders and treatment combinations of trials 1 and 2 which are followed by the same letter did not differ significantly. Comparisons of values for third-order and fourth-order roots were not made.

TABLE 4-9. The influence of an introduced composite of fungi and bacteria on the relationship between infection of Hicks cultivar of tobacco by Phytophthora parasitica var. nicotianae and total lengths of roots after 14 days of plant growth in raw or autoclaved field soil artificially infested with propagules of the pathogen

				Total Length (cm)				
				Root Order				
Trial	Soil	Antagonist[a]	Chlamydospores/g Soil	1[e]	2	3	4	Seedling
1	Autoclaved	-[b]	0	64.9x[c,d]	8.2x	1.6	0.0	74.7
			50	62.6x	7.5x	1.2	0.0	71.4
		+	0	62.5x	5.5x	1.1	0.0	69.1
			50	54.0x	8.7x	1.2	0.5	64.4
	Raw	-	0	110.6x	16.7x	1.4	0.5	129.2
			50	55.5x	9.9x	1.0	0.0	66.4
		+	0	112.4x	22.3x	2.4	1.2	138.3
			50	61.6x	11.7x	3.4	0.2	76.9

TABLE 4-9. Continued

Trial	Soil	Antagonists	Chlamydospores/g Soil	Total Length (cm) Root Order 1[a]	2	3	4	Seedling
2	Autoclaved	-[b]	0	96.8x[c,d]	17.9y	2.5	0.4	117.6
			50	83.9x	10.5x	1.3	0.6	96.3
		+	0	129.1y	19.8y	1.7	0.0	150.6
			50	79.2x	15.9x	1.7	0.4	97.2
	Raw	-	0	84.0y	11.1x	1.6	0.0	96.7
			50	64.9x	8.0y	1.2	0.0	74.1
		+	0	99.6x	15.9y	1.8	0.3	117.6
			50	85.1x	11.2x	1.4	0.6	98.3

[a] Root orders are as defined in the morphometric root analysis system (21).

[b] - = composite of antagonists was not added to soil; + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and 10^5 bacterial cells of Pseudomonas putida were added per gram of raw or autoclaved soil.

[c] Noninfested soil values are the averages of total lengths of elements per root order of three healthy seedlings; infested soil averages are from six infected root systems.

[d] Values within corresponding root orders and treatment combinations of trials 1 and 2 which are followed by the same letter did not differ significantly; Comparisons of values for third-order and fourth-order roots were not made.

TABLE 4-10. The influence of an introduced composite of fungi and bacteria on the relationship between infection of Hicks cultivar of tobacco by *Phytophthora parasitica* var. *nicotianae* and the average lengths of roots after 14 days of plant growth in raw or autoclaved field soil artificially infested with propagules of the pathogen

Trial	Soil	Antagonists	Chlamydospores/ g Soil	Mean Root length (cm)/ Root Order			
				1[a]	2	3	[illegible]
1	Autoclaved	-[b]	0	1.7[c]	1.6	1.6	[illegible]
			50	2.2	1.9	1.2	[illegible]
		+	0	2.2	1.2	1.1	[illegible]
			50	2.1	1.6	0.9	[illegible]
	Raw	-	0	2.2	2.5	1.3	[illegible]
			50	2.1	2.7	1.0	[illegible]
		+	0	2.2	2.6	1.0	[illegible]
			50	2.2	2.9	1.5	[illegible]

TABLE 4-10

Trial	Soil	Antagonists	Chlamydospores/g Soil	Mean root length (cm) Root Order 1[a]	2	3	4
2	Autoclaved	-[b]	0	2.1[c]	1.6	1.3	0.4
			50	2.3	1.8	0.9	0.5
		+	0	2.0	2.3	1.7	0.0
			50	2.1	1.9	0.9	0.6
	Raw	-	0	2.1	2.4	1.6	0.0
			50	2.5	3.1	1.1	0.4
		+	0	2.2	2.7	1.6	0.3
			50	2.5	2.3	1.2	0.0

[a] Root orders are as defined in the morphometric root analysis system (21).

[b] - = composite of isolates was not added to soil, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and 10^5 bacterial cells of Pseudomonas putida were added per gram of raw or autoclaved soil.

[c] Noninfested soil values are the averages of mean lengths of elements per root order of three healthy seedlings, infested soil averages are from six infected root systems.

Figure 4-1. The relationship of population density of Phytophthora parasitica var. nicotianae to time in autoclaved field soil not amended (○—○) or amended (●— —●) with a composite of antagonists, and in raw field soil not amended (△— △) or amended (▲— —▲) with the composite. The composite of antagonists was comprised of propagules of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and Pseudomonas putida. Soils were infested initially with 50 chlamydospores of the pathogen per gram of soil.

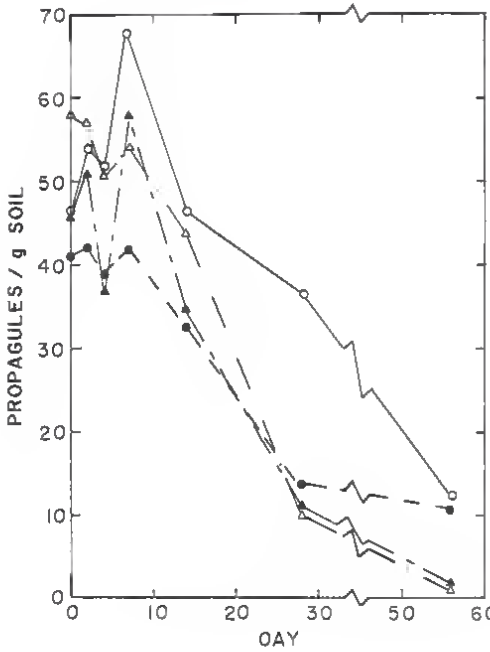

PROPAGULES / g SOIL
70
60
50
40
30
20
10
0
0
10
20
30
50
60
DAY

Figure 4-2. The relationship of population densities of Trichoderma harzianum (○—○), Aspergillus carbonarious (△— △), A. terreus (●—●), and Penicillium steckii (▲— ▲) to time in raw field soil amended with propagules of these organisms and Pseudomonas putida and infested with propagules of Phytophthora parasitica var. nicotianae.

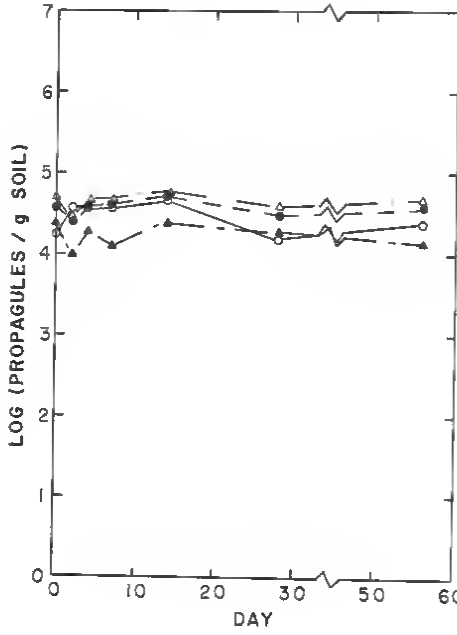
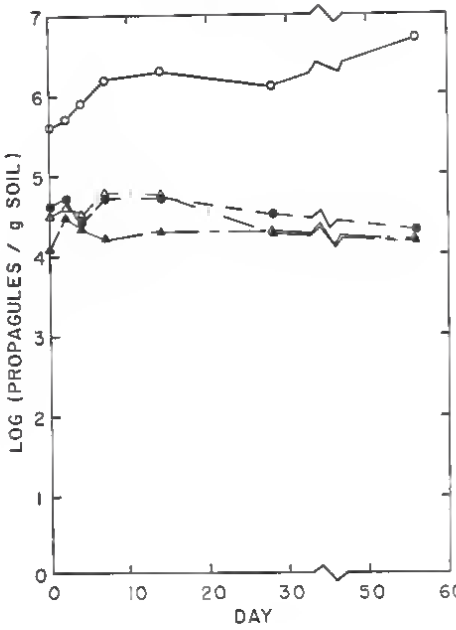

LOG (PROPAGULES / g SOIL)
7
6
5
4
3
2
1
0
0
10
20
30
50
60
DAY

Figure 4-3. The relationships of population densities of Trichoderma harzianum (○—○), Aspergillus carbonarius (△— △), A. terreus (●— ●), and Penicillium steckii (▲— ▲) to time in autoclaved field soil amended with propagules of these organisms and Pseudomonas putida and infested with propagules of Phytophthora parasitica var. nicotianae.


LOG (PROPAGULES / g SOIL)
DAY
0
1
2
3
4
5
6
7
0
10
20
30
50
60

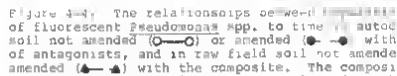


Figure 1-4. The relationships between [illegible] of fluorescent Pseudomonas spp. to time in autoclaved field soil not amended (O—O) or amended (●—●) with a composite of antagonists, and in raw field soil not amended (△—△) or amended (▲—▲) with the composite. The composite of antagonists was comprised of propagules of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii, and Pseudomonas putida. Soils also were infested with propagules of Phytophthora parasitica var. nicotianae.

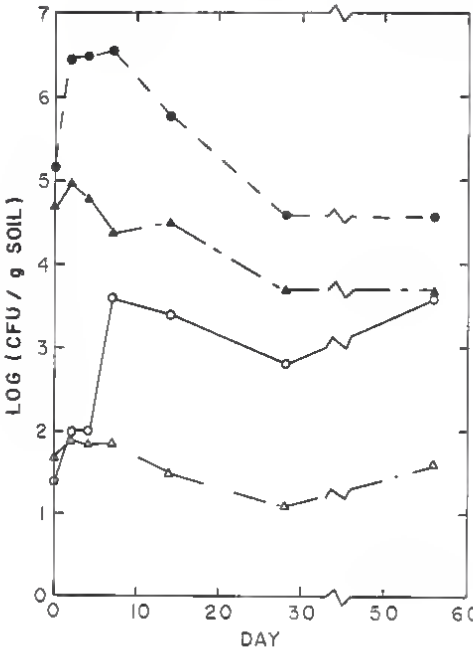

LOG (CFU / g SOIL)
0
1
2
3
4
5
6
7
0
10
20
30
50
60
DAY

TABLE 4-11. The influence of an introduced composite of fungi and bacteria on development of black shank in the susceptible tobacco cultivar Hicks grown in soil infested initially with 5 chlamydospores of Phytophthora parasitica var nicotianae per gram of soil[a]

Soil	Antagonists	Disease Development[b] t_{10}	t_{10-50}	t_{10-90}	Mortality at Harvest (%)[c]
Autoclaved	-[d]	25	27	53	100
	+	26	28	—[e]	100
Raw	-	--	--	--	14
	+	--	--	--	7

[a] Data reported are based on means of three trials.

[b] Days required to attain 10% plant death (t_{10}) or for mortality to increase from 10 to 50% (t_{10-50}) or from 10 to 90% (t_{10-90}) as interpolated from linear regression analyses.

[c] Plant mortality with 15 plants per treatment 90 days after planting in the greenhouse.

[d] - = soil was not amended with the composite of isolates, + = 10^5 conidia of each of Trichoderma harzianum, Aspergillus terreus, A. carbonarius, Penicillium steckii and 10^7 bacterial cells of Pseudomonas putida were added per gram of raw or autoclaved soil.

[e] — = insufficient disease to estimate value.

Discussion

Attempts were made to amend soil with a composite of fungi and bacteria which would preclude infection of tobacco by F. parasitica var. nicotianae through rapid colonization and occupation of infection sites within root systems. The extent of colonization of the rhizospheres and root surfaces of Wicks tobacco and of non-rhizosphere soils by these competitors varied between soil ecosystems. Within the rhizospheres and within non-rhizosphere soil of the ecosystem with amended, autoclaved soil, for example, population densities of T. harzianum increased to levels greater than those initially established. Similar increases in densities of fluorescent Pseudomonas spp. were observed in these regions of this ecosystem of one trial. In all regions of the ecosystem with amended, raw soil, these and all other introduced species never attained densities established initially (1×10^5 propagules per gram of soil). Population densities of introduced microbial species associated with root surfaces could not be compared with initially established densities in soil because estimates of those densities were made in relation to unit dry weight of roots. However, relative densities of introduced species varied in a manner similar to those observed within surrounding rhizosphere soil.

With the ecosystem with amended, autoclaved soil, T. harzianum and P. putida were the only introduced species capable of taking advantage of nnoccupied substrata. Although it was impossible to identify all fluorescent Pseudomonas spp. recovered from soil, it is likely that most were derived from the isolate added initially. Within this ecosystem, conidia of T. harzianum must have germinated readily. Subsequent rapid colonization of non-rhizosphere soil and root systems of tobacco by this species may have limited the ability of the other introduced fungal species to colonize these regions. Large populations of fluorescent Pseudomonas spp. also may have hindered colonization by introduced fungi. Reduction in root surface colonization by soilborne fungi in association with fluorescent Pseudomonas spp. have been demonstrated by Kloepper and Schroth (43, 44).

The lack of increases in densities of introduced fungi and bacteria within the ecosystem with raw soil is difficult to interpret. In non-rhizosphere soil the fairly constant population densities of fungal species may be related to fungistasis and antagonistic interactions with other ecosystem components. Similar mechanisms may have limited increases in populations of fluorescent Pseudomonas spp. The influences of fungistasis on populations of soilborne fungi in general have been discussed by Lockwood (49). Stainer and Lockwood (81) have reported correlations between the sizes of fungal spores and sensitivities to

fungistasis. The relatively small [illegible] typical, produced by the fungal species used in this study likely would have been quite sensitive to such influences. Support of this probability comes from investigations by Papavizas et al. (69) and Lewis and Papavizas (46) in which proliferation of Trichoderma spp. and other genera introduced into soil as conidia was limited by a lack of available nutrients. These authors found that chlamydospores and mycelium of various fungi added to soil with an attached food base were much more successful in proliferating within raw soil amidst a stable microbial community. Unfortunately the influence of an increased food base on germination of conidia in raw soil was not examined.

It is not likely that all conidia introduced into raw soil remained dormant or died. It may be that at any point in time only a proportion of the spore population germinated and began to grow. Such low percentages of spore germination have been reported for T. harzianum and other Trichoderma spp. and Gliocladium spp. (5). The fate of actively growing organisms is uncertain although it would be expected that actively growing hyphae would be susceptible to various types of antagonistic interactions with other soilborne microorganisms.

Whether amendment of soil with nutrients would have improved the efficacy of introduced organisms in reducing infections of roots by F. parasitica var. nicotianae is questionable. The goal in the present investigation was not

to establish high densities [illegible]
non-rhizosphere soil, but rather the intention was to
establish a stable community of microorganisms of high
density in close association with roots of tobacco. It has
been suggested that fungistasis might be reduced within the
rhizosphere in response to nutrients released from root
surfaces (14, 49). It may be that only a portion of
introduced conidia need have germinated under such
influences for the introduced fungi to have become
established around roots. Similarly a proportion of the
population of introduced bacterial cells may have become
active under the influences of released nutrients in the
vicinity of roots. Intense antagonistic interactions within
this region may have limited development of mycelium,
further spore germination, and bacterial cell division,
thus, these interactions may have limited the development of
large populations of introduced microorganisms

The increases in densities of fungi, as determined by
plate counts, observed in association with surfaces of
first-order roots of tobacco grown in amended soils
contrasted sharply with the lack of influence of amendment
on surface coverage by fungal hyphae, as determined by
direct microscopic observation. However, the lack of
alterations in surface coverage associated with
introductions of the particular fungal species in this study
agrees with previous observations related to root surface
colonization. The species utilized in the present study

were [illegible] od dominant organisms o[illegible] various hosts in several earlier studies (31, 39, 42), rather these species occurred predominantly within rhizosphere soil. In most of the earlier investigations, roots were rinsed for 20 min prior to plating to ensure removal of all but vegetative fungal structures. Roots of tobacco, however, were rinsed only for 1 min prior to removal of surface organisms using glass beads. Estimates of high densities of fungi derived from platings of root surface suspensions in the present study likely resulted from colonies which developed either from non-germinated conidia or coelia which had germinated but produced only limited mycelial growth. Such contrasts in estimates of populations by the two techniques emphasize the problems associated with interpretations of biological function from population estimates made from plate counts, the forms of organisms must be considered.

The general patterns of population dynamics of P. parasitica var. nicotianae in amended or non-amended, non-rhizosphere soil were similar to observed reductions over time in population densities of this pathogen in other field soils (38). However, populations of the pathogen declined more rapidly with time in the soil employed in this study than in soils tested in other work. The influence of reductions in densities of this pathogen in non-rhizosphere soil on long term development of tobacco black shank is uncertain. In particular, it is uncertain as to how much

populations of the pethogen would have to be reduced to significantly reduce disease development. Densities of the pethogen after 56 days in amended and non-amended soils varied between approximately 2 and 12 propegules per gram of soil. Reductions in population densities to as low as 0.1 propegules per gram of soil likely would not reduce disease incidence, Kannwischer and Mitchell (40) showed that this density of inoculum was sufficient to cause 50% infection and mortality of tobacco. Densities of P. parasitica var. nicotianae as low as 0.01 propegules per gram of soil have been observed in a tobacco field prior to planting (20). Mortality of tobacco was observed within that field later in the growing season. Reductions of inoculum densities of this pethogen in non-rhizosphere soil to levels sufficient to reduce disease development are not likely to be achieved economically.

The introduction of a composite of microorganisms into soil may have reduced somewhat the colonization of tobacco root systems by P. parasitica var. nicotianae. Although significant reductions in the average numbers of infections per infected seedling were not observed in association with amendment of soil, the trends in this direction were encouraging. Significant reductions in the average number of infections per infected seedling and efficiency of inoculum for infection might have been attained either by reducing the initial density of inoculum of the pethogen or by increasing the period of plant growth in infested soil.

The extension of period of plant growth, however, likely would produce difficulties associated with the logistics of handling much larger root systems and maintaining adequate replication.

The values of root growth parameters associated with infected tobacco plants did not differ significantly from values of those parameters associated with non-infected plants within the same soil ecosystem. Nevertheless, the numbers and total lengths of first-order and second-order roots of infected seedlings tended to be less than corresponding numbers and lengths of those roots of healthy plants. Such differences may have become significant with longer periods of plant growth as infected roots became necrotic. The trends towards alterations of patterns of root growth appeared to be the result of infection rather than colonization by the introduced competitors. This conclusion is supported by the observed significant influence of infection on root system development when averaged over amended and non-amended, raw and autoclaved soils. Soil amendment with the composite, as an independent effect, was not associated with significant alterations in root system development. It was not surprising that average total root lengths per seedling varied in association with treatment combinations in a manner similar to that for first-order and second-order roots. Since more than 80% of the total lengths comprised first-order roots alone, it would be expected that changes in total seedling root length

would be strongly influenced by [illegible] this root order.

As in previous infection trials [illegible], values of parameters of root growth at times fell outside the 95% confidence intervals for expected values of these parameters after 14 days of tobacco growth (cf. Table 2-8). Reductions in root growth most commonly were associated with infection of tobacco plants by P. parasitica var. nicotianae. However, values of parameters of healthy seedlings grown in autoclaved soil in trial 1, at times, also fell outside these intervals. Root growth of healthy seedlings in raw soil always fell within the confidence intervals. Whether a causal role for infection may be assumed as regards reduced root growth is uncertain. Further evaluations of this relationship after an extended period of plant growth in raw soil, in particular, are required before conclusions may be drawn.

Whether alterations in patterns of development of root systems associated with infection in the field may be detected is uncertain. Since inoculum densities of P. parasitica var. nicotianae in the field initially are very low, extensive growth of root systems would be expected before contact with pathogen propagules would occur. Early infections of tobacco in such a setting likely would be sporadic within individual root systems. Alterations of root growth probably would occur on a local basis around points of infection in association with progressive root

tissue colonization emanating from such infection points. Ultimately necrosis would limit growth.

Further suggestions of efficacy of introduced fungi and bacteria for reduced infection of tobacco by P. parasitica var. nicotianae and subsequent black shank development were derived from competition trials in the greenhouse. Although significant reductions in mortality were not observed in association with amendment of soils with microbial isolates, the trends were in that direction in both soil ecosystems. The lack of significant reductions in infection may have been the result of simultaneous amendment and infestation of soil with the composite and the pathogen at the time of planting. Further reductions in mortality may have been attained by colonization of roots with competitors prior to transplant of seedlings into infested soils. In the case of simultaneous amendment and infestation of autoclaved soil, sufficient coverage by competitors of sites susceptible to infection may not have been attained in a large proportion of the host population. Equivalent values of t_{10} and t_{10-50} observed in amended and non-amended autoclaved soils supported such a possibility. Root systems of a smaller proportion of the host population were protected sufficiently by rapid colonization by introduced isolates as reflected by a lack of increase in mortality sufficient to estimate t_{10-90} in amended, autoclaved soil. It could not be ascertained whether colonization of tobacco root systems by antagonists reduced or delayed initial infections, or

reduced the rate of progressive root tissue colonization in raw soil coverage of infection sites may have been attained much earlier as suggested by the significant reductions in mortality of tobacco grown in these soils from levels of mortality of plants grown in autoclaved, infested soils. Mortality of tobacco grown in raw soil amended with the composite was not reduced significantly from mortality of plants grown in non-amended, raw soil. Thus, it appears that other microorganisms within this ecosystem also were involved in protecting tobacco root systems from infection by the pathogen.

The composite of fungi and bacteria evaluated within these trials was comprised of organisms selected for rapid colonization of root systems of tobacco. It is not known what other antagonistic traits, if any, might have been carried with the isolates introduced into soil. Improvements in the performance of a composite might be obtained by further screening of selected isolates for other traits of antagonism or by selections of isolates known to colonize additional critical regions of the ecosystem. Additionally, the efficiency of colonisation of root systems by introduced organisms might be improved by the use of more appropriate propagules of these organisms. Such considerations might be necessary especially for manipulations of microbial populations in ecosystems with raw soil

CHAPTER V
SUMMARY AND CONCLUSIONS

A number of soilborne fungi and bacteria have been evaluated for antagonism against Phytophthora spp. by the use of in vitro assays. Many of these microorganisms have proven to be antagonistic to pathogens either through the production of antibiotics or through hyperparasitism (51, 67). Evaluations of these antagonistic isolates for control of diseases caused by Phytophthora spp have been few and empirical in nature. Trials have involved measurements of disease development in host plants grown in soil or other media amended with individual antagonistic isolates. Little insight has been provided as to reasons for the success or failure of particular isolates in reducing development of disease.

In the present investigation of black shank of tobacco (Nicotiana tabacum L.), an analytical approach was utilized to evaluate pathosystem behavior. The system was broken into components including populations of the pathogen, host roots, and surrounding soilborne microorganisms. The behaviors of individual components and interactions between them were examined. It was of particular interest to evaluate the ability of an introduced composite of microorganisms to colonize sites susceptible to Phytophthora

parasitica [illegible] nicotianae (Breda de Haan) Tucker within root systems of tobacco and to protect such sites from infection.

To quantify component behaviors and interactions, assays were developed which were rapid and repeatable. Development of root systems of tobacco was analyzed by the use of the morphometric root analysis system (21). This system was appropriate for quantification of the development of root tissues in relation to susceptibilities to infection by P. parasitica var. nicotianae. Initial trials conducted in a plant growth room revealed that the development of root systems of the susceptible tobacco cultivar, Hicks, was similar to that of the resistant tobacco cultivar, Speight G-28, during 15 days of plant growth in both stable and disrupted soil ecosystems represented by raw and autoclaved soils, respectively. The numbers and total lengths of various root orders did not differ significantly between cultivars. However, it appeared that with additional plant growth, differences may have become apparent. Ultimate structures of root systems appeared to be determined partially by the rates of root multiplication and extension.

Numbers and total lengths of elements of first- and second-order roots increased exponentially over time. Such patterns of increase are typical of those which have been observed for other plants during early stages of growth (21, 35, 58). At later stages of plant development, the rates of root system development actually may decrease in association

with shifts from vegetative to reproductive phases [illegible] (35).

The development of root systems of tobacco was controlled to variable extents for the two cultivars examined. Growth of roots of Speight G-28 tobacco, in particular, was very consistent over trials. Root growth of Hicks tobacco was more variable over trials, possibly in relation to greater variability in sizes of seedling transplants. Greater control of the development of root systems of tobacco might be attained if plants are initiated from seed screened for uniformity.

The patterns of early root system development were not altered significantly by infection with P. parasitica var. nicotianae. Infected roots of tobacco could not be distinguished by appearance from noninfected roots; experiments were halted before extensive root tissue colonization by the pathogen and necrosis had occurred. Despite the lack of significant alterations in root growth associated with infection, there was a trend towards reduced numbers and lengths of roots of infected plants as compared to healthy plants. With time, differences may have become apparent.

Differences in the development of root systems in association with infection by P. parasitica var. nicotianae in field situations would be more difficult to detect. In that setting contact between roots of tobacco and propagules of the pathogen initially would occur infrequently because

inoculum densities of the pathogen are extremely l[illegible]. During the period of initial root tissue colonization by the pathogen, any alterations in root growth likely would be evident locally in association with points of infection. Such alterations would be difficult to perceive in relation to the entire plant root system.

The incidences of plant infection and average numbers of infections per infected seedling were similar for resistant and susceptible tobacco cultivars in stable and disrupted soil ecosystems. The efficiencies of inoculum for infection were very low and thus implied infrequent successful contact between susceptible root tissues and propagules of the pathogen during the 14 days of their interaction. Results of point inoculation trials suggested also that infection of the most susceptible root tissues may not be completely efficient even when such tissues are in contact with propagules of the pathogen. Within intact root systems, the zones just behind root tips were most susceptible to infection by F. parasitica var. nicotianae; over 73% of these tissues were infected when inoculated with small numbers of zoospores. Percentage of infection declined rapidly with increasing maturity of root tissues at points of inoculation. The small number of zoospores applied as inoculum at individual points represented the situation likely to be encountered in normal field situations. Inoculum available at sites of infection likely would be restricted to very few chlamydospores, sporangia,

[illegible] released from sporangia. Prior to [illegible] most investigations had focused on demonstrating aggregation, encystment, and infection by zoospores [illegible] root tips by immersion of roots into dense suspensions of zoospores (26, 29, 60, 65).

Comparisons were made as well between patterns of development of microbial communities associated with root systems of Hicks tobacco developing in stable and disrupted soil ecosystems. Patterns of colonization by soilborne microorganisms were complex and dynamic. Generally the diversity of organisms associated with tobacco roots was greater in raw soil than in autoclaved soil. This was obvious particularly in terms of the densities and types of fungi which colonized root systems in the two soil ecosystems. Colonization of surfaces of first-order roots by fungi was more extensive in the stable soil ecosystem than in the disrupted system; the average length of fungal hyphae and density of hyphae at occupied points was greater in association with roots in the former ecosystem. Colonized regions along root surfaces were more patchy or aggregated within the disrupted soil ecosystem. Patterns of colonization along root surfaces remained fairly constant over time, as would be expected, since samples at each date were taken from tissues of similar physiological age.

A group of fungi and bacteria which colonized roots of tobacco rapidly and stably in raw soil was evaluated for its ability to compete with *P. parasitica* var. *nicotianae* for

[redacted] susceptible to infection by the pathogen. The composite of organisms comprised one randomly selected isolate each of Trichoderma harzianum, Aspergillus carbonarius, Aspergillus terreus, Panicillium steckii, and Pseudomonas putida. Amendment of soil with propagules of this composite was associated with increases in numbers of recoverable propagules of total fungi and fluorescent Pseudomonas spp. within rhizospheres and at root surfaces of tobacco and in non-rhizosphere soil within stable and disrupted soil ecosystems as determined by soil plating. A lack of observable alteration in extensiveness and density of root surface colonization by fungal hyphae suggested that most propagules of the composite fungi in that region consisted of recently germinated or non-germinated conidia. In competition trials amendment of infested soils with the composite of isolates was not associated with reductions in numbers of infections observed per infected Hicks tobacco seedling; similarly, incidence of plant infection was not reduced in association with soil amendment. Despite the lack of significant reductions in infections associated with soil amendment, trends in this direction were observed. Again, with sufficient time, significant differences may have become evident. As in earlier infection trials, the development of root systems of Hicks tobacco was not altered significantly in association with infection by the pathogen within individual soil ecosystems. Similarly, amendment of soil with the composite of microorganisms did not influence root system development.

Popnlation densities of P. parasitica var nicotianae dsclinsd rapidly over time in raw and autoclavsd soils smended or not smended with the composite of mioroorganisms. Densities of the pathogen did not differ significantly betwaen ecosystems at any smepling date. The importance of redoctions in densities of the pathogen in non-rhizosphere soil to disease dsvelopment in the field is uncertain. Kannwischer and Mitchell (40) reported disesse incidance and eortality of 50% whan tobacco plants were grown in soil infestad with approximatsly 0.1 chlaeydospore af ths pathogen per gram of soil. Redoctions of densities of the pathogen to lsvels lower than this by soil amendment may nat occur.

In greenhonse trials amendment of infested soil with ths composite of isolates resulted in decreased mortality of tobacco after 90 days of plant growth, differences, however, were not significant. Lack of control of black shank in this trial eay have been associated with simultaneous infestation of soil with ths pathsgen and amendeent with the composits. Occupation of infection sites by competitors may not have bsen sufficient to prsclude ths few infections by the pathogen which wonld have bean required to kill the tobacco plants. Greatar control eay have been attained if tobacco root systeas had bean colonized with the composita prior to transplanting into infastad soil.

The information provided in this series of experiments has laid the groundwork for further analytical evaluations of interactions between components of the black shank pathosystam. The use of the morphometric model of root system development provided a method with which to evaluate availability of root tissues to infection by P. parasitica var. nicotianae over time. The use of Lloyd's indices of mean crowding and patchiness provided a means to evaluate colonization of root surfaces by fungi. Yet more information is needed in regards to colonization of tobacco roots by P. parasitica var. nicotianae after initial infection. The development of such approaches for analysis of pathosystem behavior in situ is important. By utilizing such approaches, comprehension of pathosystem function may be increased through reductions of artifacts associated with in vitro approaches.

The composite of microorganisms analyzed in these studies was selected strictly on the basis of rapidity of site occupation. Occupation of sites to reduce infection by P. parasitica var nicotianae appeared to be partially successful in stable and disrupted soil ecosystems. It is not known if these isolates possessed other traits of antagonism as well. A next logical step would involve further screening of isolates which colonize roots rapidly for expressions of additional antagonistic traits. It would be worthwhile as well to select organisms which colonize root surfaces more effectively to more completely occupy important niches within the ecosystem.

With the foundation that has been laid, this system may be used to investigate the contributions of numerous environmental factors and cultural practices to pathsystem behavior. It should be helpful in isolating components and component interactions which are influenced by such treatments.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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